

THE METABOLISM OF HISTAMINE  
AND ITS ROLE IN CERTAIN PATHOLOGICAL PROCESSES

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BY

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Histamine may produce signs and symptoms not only by its general effects throughout the body, but also by its local effects at sites of release. Investigation of the role of histamine in pathological conditions will depend upon knowledge of the rate and extent of its metabolism in the intact animal, and of concentration of the local tissue concentrations and actions in particular conditions. In the present experiments, these aspects of the metabolism and pathological characters of histamine have been investigated with reference to the damage induced by histamine during irradiation, and with reference to local gastric damage.

In these conditions, the physiological actions and pathological effects which could be attributed to histamine are not dissimilar. There is evidence that changes occur in the physiological concentrations of histamine following irradiation, and that pathological lesions are sensitive to infusion of histamine. Such points are believed to be relevant during the study of the role of histamine in the pathogenesis of the damage induced by histamine during irradiation.

## CHAPTER 1

### THE METABOLISM AND FUNCTION OF HISTAMINE

At the Ciba Foundation Symposium on Histamine, many aspects of the cellular metabolism, tissue distribution and physiological function of histamine in particular tissues were discussed. It became clear that little was known about the physiological dissemination of exogenous or endogenous histamine in the intact animal. At the end of the symposium Feldberg suggested that the mode of action of histamine could form the subject for another symposium (Ciba Foundation Symposium, 1956). Perhaps he made this suggestion because references to its function in pathological conditions had been scanty; yet the anti-histamines are well used therapeutic weapons of the present day, employed for treatment of conditions attributed to the pathological action of histamine. It is clear that the body's control of the dissemination of histamine through the tissues will determine the extent to which histamine can produce physiological, and indeed, pathological effects. In particular areas of the body, circumstantial evidence suggests that histamine may play a part in producing local changes and possibly pathological conditions. Reference was made to this evidence at the Ciba Symposium by Perry when he referred to the possible function of histamine in the skin and by Whelan when he referred to its vasodilator properties. Code has accused endogenous histamine of being responsible for the production of gastric ulcers, since he has stated that it is undoubtedly responsible for acid gastric secretion, (Ciba Foundation Symposium, 1956), and both he and other workers have said that acid is responsible for the production of ulcers.

Histamine may produce signs and symptoms not only by its general effects throughout the body, but also by its local tissue effects. Investigation of the role of histamine in pathological conditions will depend upon knowledge of the rate and extent of its metabolism in the intact animal, and on examination of its local tissue concentrations and actions in particular conditions. In the present experiments, these aspects of the metabolism and pathological characters of histamine have been investigated with reference to the damage sustained by animals during irradiation, and with reference to local gastric damage.

In these conditions, the physiological actions and pathological effects which could be attributed to histamine are not unconnected. There is evidence that changes occur in the normal blood concentrations of histamine following irradiation, and that irradiated animals become more sensitive to infusion of histamine. Toxic products are believed to be liberated during irradiation from the alimentary canal; it is possible that one of these products could be endogenous histamine. The alimentary



canal contains high concentrations of histamine, which, in the stomach at any rate, it has been suggested can play a pathological role. Irradiation causes a break-down in the continuity of skin surfaces, and tissue damage in the intestine. Gastric ulceration can be defined as a break-down in the continuity of the gastric mucosa associated with local tissue damage. It seems unlikely that such break-downs in the continuity of surface coverings, and local tissue damage are completely unconnected; it is possible that the local release of histamine could play some part in their initiation. This suggestion is supported by the observation that exogenous histamine can produce local tissue damage in the stomach and profound local changes in the skin. Why should endogenous histamine in abnormal circumstances not play a similar role?

The experiments described in this thesis attempt to find an answer to this question. The pharmacological actions of histamine are well-known, and the details of its tissue metabolism have been extensively investigated. The use of this knowledge and the development of further experimental methods have enabled part of the answer to the question to be obtained. However, in all research, the answer to any question always provides several further problems. The way in which the problems, which have been raised in the present investigations, might be solved is suggested in the discussion in each chapter. In the final chapter, the initial experiments which have been performed in an attempt to find an answer to one of the problems have been briefly discussed.

As on the stomach of the guinea-pig, have been investigated, but no experimental methods have been used in common for all of these investigations. However, some techniques have been employed more than once in the different experiments. It has, therefore, been considered justifiable for the sake of brevity, and in order that the main argument of the work should not be interrupted by the insertion of experimental details, to group the experimental procedures together in one chapter. Reference is made where necessary in subsequent chapters to the methods and procedures which have been used.

## CHAPTER 2

## EXPERIMENTAL METHODS

The methods of assay and procedures used in the experiments described in the following chapters, include a wide range of pharmacological, biochemical and pathological techniques. It has been necessary to use this extreme range of techniques because the physiological and pathological aspects of the actions of exogenous and endogenous histamine have been investigated not only in general in tissues throughout the body, but also in more detail in tissues where special techniques are required, such as in the kidneys, skin and stomach. The metabolism and distribution of histamine in the tissues of the normal rat, the effect of irradiation on the metabolism of endogenous histamine in the rat, and the mechanism of production of pathological effects on the stomach of the guinea-pig, have been investigated, but no experimental methods have been used in common for all of these investigations. However, some techniques have been employed more than once in the different experiments. It has, therefore, been considered justifiable for the sake of brevity, and in order that the main argument of the work should not be interrupted by the insertion of experimental details, to group the experimental procedures together in one chapter. Reference is made where necessary in subsequent chapters to the methods and procedures which have been used.

18/50, described in Chapter 3, either by intraperitoneal injection or by stomach-tube. Doses are expressed in terms of histamine.

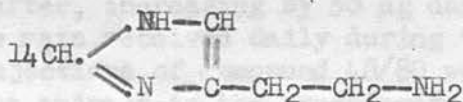
### ANIMALS.

**Rats:** All the experiments described in Chapters 3 and 4 were performed on male albino rats of the Wistar strain weighing 100 - 200 g. The rats were fed on cake and water ad libitum until the beginning of the experiments. In the experiments which continued during several days, there was no limitation of food intake.

**Guinea-pigs:** Male guinea-pigs with a weight range of 300 - 500 g were used for the experiments on gastric physiology and pathology described in Chapter 5. Animals with weights at the upper end of the range were used in the experiments in which it was necessary to pass the stomach-tube frequently. The animals were fasted for 15 hours before the start of the experiments although water was given ad libitum during this period.

### ADMINISTRATION OF DRUGS.

**Histamine:** Radio-active histamine in the form of bihydrochloride was administered by intravenous injection in a dose of 5 mg/kg in the experiments described in Chapter 3. The histamine was marked with Cl4 in position 2 in the imidazole nucleus.



The radioactive histamine was prepared from potassium thiocyanate by the method of Frazer & Raphael (1952) by the Radiochemical centre at Amersham, England. Two different samples were used, the specific activities of which were 4.176 and 1.48 mc/mm. The first sample was diluted with non-istopic histamine in the following proportions: crystallized histamine bihydrochloride Cl4 96.2 parts + 3.8 parts histamine bihydrochloride "Roche". The second sample was used undiluted. Doses are expressed in terms of the bihydrochloride.

Histamine was given in the form of histamine acid phosphate to the rats which had previously received compound

48/80, described in Chapter 3, either by intraperitoneal injection or by stomach-tube. Doses are expressed in terms of histamine base.

Histamine acid phosphate suspended in 4.5% (w/v) white beeswax in arachis oil (Williams, 1951) was given by intramuscular injection to investigate gastric function and pathology as described in Chapter 5. Doses are expressed in terms of histamine acid phosphate. A dose of 10 mg/kg was given for stimulation of maximum secretory response, and a dose of 20 mg/kg was administered for production of gastric ulceration. The injection was given into the muscles of the thigh, and after injection, the site was gently massaged for about 5 seconds. The larger dose was given 30 minutes after intraperitoneal injection of anthesisan 150 µg/kg which prevented collapse or death from histamine shock.

Methacholine: This was administered to guinea-pigs by intraperitoneal injection to stimulate gastric secretion as described in Chapter 5. Doses of 0.02 mg/kg were given at 10 minute intervals over a period of 50 minutes. Gastric secretion was collected by stomach-tube 1 hour after the first injection of methacholine, that is 10 minutes after the last injection of the drug.

Compound 48/80: Depletion of the skin and tissue histamine of the rats in the experiments described in Chapters 3 and 4 was carried out by the intraperitoneal injection of compound 48/80 twice daily in gradually increasing doses. During the first six days, compound 48/80 was injected in the morning and afternoon, thereafter, it was injected only in the morning. It was given in the following doses, calculated in µg/100 g; on the first day, 90 + 90; second day, 120 + 120; third day, 150 + 150; fourth day, 200 + 200; fifth day, 250 + 250; sixth day, 300 + 300; and thereafter, increasing by 50 µg daily up to 550 µg/100 g, which the rats received daily during the remainder of the experiment. Injections of compound 48/80 were given for only four days to the animals in the experiments described in Chapter 3 in which the reformation of skin histamine and urinary excretion of histamine were being measured. Exogenous histamine was injected into these animals on the sixth day.

Phenylbutazone and magnesium oxide: Phenylbutazone (Butazolidin, Geigy) without added xylocaine was given by intraperitoneal injection in a dose of 200 mg/kg to the guinea-pigs in the experiments described in Chapter 5.

Three groups of guinea-pigs also received magnesium oxide pond. suspended in 2.0 ml distilled water by stomach-tube. During administration, the end of the tube was never introduced beyond the cardia, in order to ensure that the gastric mucosa was not damaged. The guinea-pigs in groups 1 and 2 received the MgO at hourly intervals, the first dose being given one hour before the administration of the phenylbutazone, and the last dose one hour before they were killed, that is, six hours after



receiving the phenylbutazone. Group 3 received the alkali at irregular intervals during a 12 hour period. The doses and times of administration of the alkali are shown in Table 1. Each group contained 8 animals.

#### EXPERIMENTAL PROCEDURES:

Removal of blood: Blood was removed at intervals after the injection of radio-active histamine into the rats, described in the experiments in Chapter 3. The samples were removed exactly at the required times from 1 - 240 minutes after the injection, by puncture of the retro-orbital plexus as described by Halpern & Pacaud (1951).

Removal of Gastric secretion: Gastric secretion was removed from fasting guinea-pigs in the experiments described in Chapter 5, according to the method described by Watt & Wilson (1957). The secretion was collected through a polythene tube in the unanaesthetised animal. The tube was introduced into the mouth while the guinea-pig was held firmly by the skin of the back of the neck with the abdomen uppermost and the neck fully extended. It was passed along the groove in the middle of the hard palate and eased gently down the oesophagus until it passed the cardia. About 6 - 8 minutes was required to remove the full volume of secretion from each guinea-pig.

Local reactions of the skin vessels: The response of normal and irradiated abdominal skin in the rat to intradermal injections of saline, histamine, and compound 48/80 was observed after intravenous injection of 0.5 ml. of 1.5% pontamine sky blue. Histamine or compound 48/80 were injected in concentrations of 1/500, 1/1000, 1/10,000, 1/20,000 and 1/50,000. Twenty hours after irradiation, pontamine sky blue was injected intravenously and immediately afterwards, 0.1 ml of one of the drugs was injected intradermally into the centre of the irradiated area and also into normal skin over the lower part of the thorax. Fifteen minutes later, the animal was killed and the skin was dissected off the abdomen and chest and mounted on a glass slide and frozen. The extent and intensity of the blueing in the normal and irradiated skin could then be compared. It was found that killing the animal 15 minutes after injection of the drugs produced optimal blueing with the concentrations used. The interval between the intradermal injections into the normal and irradiated skin was less than 30 seconds and the order of injections did not influence the results.

Exclusion of the kidneys from the circulation: A group of experiments was carried out as described in Chapter 3, on rats where the kidneys had been excluded from the circulating blood in order to observe the effect of the kidney on the metabolism of histamine. The experiments were performed simultaneously on intact animals and on animals on which an effective nephrectomy had been carried out by ligation of the renal pedicles. To avoid the effects of surgical shock, silk ligatures were placed loosely round the renal pedicles in a preliminary operation and



TABLE 1

Times of administration and doses of magnesium oxide given to the groups of guinea-pigs, together with phenylbutazone. Magnesium oxide administered by stomach-tube.

Time hours	Group 1	Group 2	Group 3
- 1	30	200	50
0	Phenylbutazone 30	200 mg/kg 200	1P1 50
1	30	200	20
2	30	200	20
3	30	200	-
4	30	200	-
5	30	200	-
6	Killed	Killed	50
7			-
8			-
9			50
12			Killed

Radiation techniques and doses: Various doses of X- and  $\gamma$ -irradiation were administered to rats in the experiments described in Chapter 4. The irradiation was given as total body radiation in the smallest dose used. Irradiation in doses greater than 1000 r was given to animals protected by lead shields or was administered by means of local applicators, in order to localize the effects to particular areas of the skin and avoid the mortality associated with large doses.

The rats received rat chow and water ad libitum preceding and following irradiation. Those which received total body radiation had no special treatment. Those which received local radiation of the abdomen were anaesthetized with nembutal at 40 mg/kg by intraperitoneal injection and their abdomens were shaved prior to irradiation. Chemical depilators were not used. Any rats with visible damage to the skin after shaving were discarded. The animals were replaced in their cages and they were randomly selected at intervals following irradiation for

the ends of the ligatures were brought to the surface of the skin on the back through the operation wound. Four or five days later, after the animals had recovered from the operation, the ligatures were tightened and secured round the renal vessels. After the experiments had been completed, post mortems were performed and it was always confirmed that the renal pedicles had been effectively ligated, and that the kidneys had been completely excluded from the circulatory system.

Hydration of rats and collection of urine: The total excretion of urine was collected for short periods after the injection of radio-active histamine by puncture of the bladder after ligation of the penis.

In the longer experiments described in Chapter 3, the urinary excretion of histamine was measured at intervals in rats which had received a water-load, using the method described by Wilson (1954a). A water-load was administered to the rats and when a diuresis had started, the urine was collected at half-hourly or hourly intervals. After a control period, histamine was given by intraperitoneal injection or through the stomach-tube and the urine was then collected for a further 3 or 4 hours depending upon the mode of administration of the histamine. The urinary histamine was assayed by diluting the urine and adding it directly to the bath containing the guinea-pig ileum. The experimental procedure for the conduction of the experiments is shown in Table 2. The normal urinary excretion of histamine was measured during the control period, and the excess excreted after administration of the histamine could then be determined. The results of these experiments are expressed as  $\mu\text{g}$  of histamine base excreted throughout the period after administration of the histamine in excess of the calculated normal excretion during the same period. All the experiments were performed on four rats at the same time; and the value for the excess normal excretion is the mean value obtained from the four animals.

Radiation techniques and doses: Various doses of X- and  $\beta$ -irradiation were administered to rats in the experiments described in Chapter 4. The irradiation was given as total body radiation in the smallest dose used. Irradiation in doses greater than 1000 r was given to animals protected by lead shields or was administered by means of local applicators, in order to localise the effects to particular areas of the skin and avoid the mortality associated with large doses.

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TABLE 2

Experimental procedure for measuring urinary excretion of histamine showing typical results from two rats which received histamine by intraperitoneal injection, and by stomach-tube. Water replacement performed by stomach-tube at hourly intervals. Histamine administered after urine collection at Time 0. Doses of histamine base: by intraperitoneal injection 150  $\mu\text{g}/\text{kg}$ ; by stomach tube 1000  $\mu\text{g}/\text{kg}$ . Excess excretion over normal after intraperitoneal injection: 4745  $\mu\text{g}$ ; and after administration by stomach-tube 1615  $\mu\text{g}$ .

Time hours	Intraperitoneal Injection		Stomach Tube		
	Urine ml	Histamine excreted $\mu\text{g}$	Urine ml	Histamine excreted: Experimental values	$\mu\text{g}$ Calculated per hour
2	-	-	-	-	-
1 $\frac{1}{2}$	7.6	1140	-	-	-
1	8.2	1230	-	-	1650
$\frac{1}{2}$	7.7	1150	-	-	-
0	8.5	1700	16.5	3300	1650
$\frac{1}{2}$	5.4	3780	-	-	-
1	3.0	2100	7.0	3185	3185
1 $\frac{1}{2}$	5.5	2310	-	-	-
2	4.0	1200	7.5	1575	1575
2 $\frac{1}{2}$	6.1	1525	-	-	-
3	5.1	1530	-	-	1690
3 $\frac{1}{2}$	-	-	-	-	-
4	-	-	13.0	3380	1690

investigation of local reactions of the skin vessels. They were investigated in groups of three at a time and so all observations were made on at least three rats and generally on groups of six or nine rats.

The radiation conditions for total body irradiation were as follows: 200 kilovoltage, 10 milliamperes, half-value layer 1.3 mm. of copper, 40 cm. focal skin distance. The exposure rate was 29 r (roentgens) per minute, estimated at the surface of the skin nearest to the applicator. Rats which were given total body irradiation received 1025 r on the skin of the back and 605 r on the abdominal skin. Each rat occupied a stall in a partitioned perspex box with walls 5 mm. thick, the ends of which were drilled for ventilation. The box was placed at the end of a 15 by 20 cm applicator. Those which received local radiation of the abdomen were anaesthetised, and were irradiated whilst wearing a 3 mm lead shield covering pelvis and chest and head with an aperture of 5 x 4.5 cm. over the abdomen. They received a dose of 1000 r to the abdominal skin. Doses of 2000 r were administered with a 3 cm. diameter circular applicator of focal skin distance 27 cm which was placed in contact with the shaved abdomen while the rat was anaesthetised. Treatment time was 34 minutes.

$\beta$ -irradiation was given by means of a 10 millicurie strontium 90  $\beta$ -ray plaque of circular area 4 sq. cm with a surface dose rate of 5260 rep/hour. This was strapped directly onto the shaved abdominal skin. The half-value depth of this  $\beta$ -radiation was 1.3 mm. of tissue and the dose to the skin surface was 3000 rep.

#### METHODS OF ASSAY:

Biological assay of histamine: Histamine was assayed biologically on the atropinized guinea-pig ileum using the automatic assay apparatus described by Boura *et al.* (1954) after extraction from the tissues, blood or urine. The extraction procedures were similar to the normal procedures with minor modifications in technique. Different procedures were used in the groups of experiments described in Chapters 3 and 4, but each procedure was used exclusively in each group and so the results within the groups are comparable.

Extraction by Code's method, (Code 1937) used in Chapter 3: The tissues were cut into slices 1  $\mu$  thick in the freezing microtome immediately after the rats had been killed by exsanguination, and were then placed in N. HCl. After boiling, the tissue was extracted by Code's method. Blood and urine were extracted according to Code's method without any modification.

Extraction by the method of Feldberg & Talesnik (1953) used in Chapter 4: Immediately after death, the abdominal skin was shaved and a sample of skin weighing about 500 mg was taken from the left side of the middle line of the abdomen. The skin sample was split from the underlying tissue on the surface of the muscle



along the natural line of cleavage, weighed and boiled in 2 ml/g of N. HCl. It was then ground with sand and subsequently treated by the method of Feldberg and Talesnik (1953). The posterior wall of the portion of the stomach lying between the pylorus and fundus, known as the pyloric part of the stomach, was removed. It was spread out, washed gently with a stream of saline, dried on filter paper and about 500 mg were weighed and boiled in N.HCl, 2 ml/g. The stomach was extracted for histamine in the same way as the skin. The mesentery was dissected from the jejunum. The first 2 cm were used for histological examination. The rest of the jejunum was split open and washed. About 500 mg of the proximal portion were weighed and immediately boiled in N.HCl and treated like the skin and stomach.

#### Radio-active Assay of histamine:

Estimation in blood: A known volume of plasma was spread in a thin layer on a glass cover-slip of 22 mm diameter and the radio-activity was measured in a Geiger-Müller counter. The activity of the blood corpuscles was measured in the same way after a known volume of the cells had been haemolysed by diluting them 1:4 in distilled water, and then exposing them on glass slides in the counter.

Estimation in tissues: The rats were killed by exsanguination and immediately afterwards, 300 - 400 mg of each tissue was cut in a freezing microtome into sections 1  $\mu$  thick and placed in an aqueous solution of Tween 80, 1/1000. The tissue suspension was placed in a thin uniform layer on cover-slips 22 mm in diameter which corresponded to the size of the window in the Geiger-Müller counter. The cover-slip was mounted in an aluminium cup and was placed at the entrance to the opening of the counter where the maximum exposure for measurement of the  $\beta$ -radiation was obtained. The radio-activity in the blood and tissues which corresponded to chemically unmodified histamine was calculated by using a layer of tissue of uniform thickness for absorption of the  $\beta$ -rays. The optimum depth of the layer was established by adding known quantities of radioactive histamine to samples of the tissues of various depths. Under these conditions, and with the apparatus which was used (Tracerlab), 10% of the radio-active histamine was recovered. In the results, the  $^{14}\text{C}$  radio-activity in the tissues has been expressed as  $\mu\text{g}$  per gram of histamine bihydrochloride, and is referred to as "radio-active histamine".



Blood coagulation time: Blood was obtained from the tail vein after the tail had been held in warm water for 2 - 3 minutes. The coagulation time was estimated by the method of Griffith and Farris (1942).

Analysis of gastric secretion:

Volume and electrolytes: The total volume and volume of solid material in the secretion were recorded after centrifuging. The concentrations of free and total acid were measured by titration of samples of the secretion of 0.5 or 1.0 ml. volumes, against 0.04N. NaOH, using Topffer's reagent and phenolphthalein as indicators. The concentration of sodium and potassium were measured by the method of Conway, (1935). The results are expressed in milli-equivalents per litre of gastric juice.

The peptic activity of the gastric secretion: This was measured by colorimetric determination of the tyrosine released after incubation of the gastric secretion with plasma substrate according to the method of Hunt (1948) as modified by Sircus (1954) for measurement of peptic activity in urine. The results are expressed in units of pepsin per 1000 ml gastric secretion. One unit of pepsin is the amount of enzyme releasing the colorimetric equivalent of 1 mg of L-tyrosine from 0.1 g of plasma protein substrate in 15 minutes at 37°C. In the method of Sircus, the incubation period was 2 hours; in the present method incubation for 15 minutes was adequate on account of the high peptic activity of the gastric secretion.

PATHOLOGICAL AND HISTOLOGICAL TECHNIQUES.

Macroscopic and microscopic examination of gastric mucosa: The guinea-pigs described in Chapter 5, which had received phenylbutazone or histamine, were killed by a blow on the head at intervals from 2 - 24 hours after receiving the drug. The stomach and duodenum were removed intact and the gastric contents were withdrawn through the cardiac orifice, care being taken not to damage the mucosa during this process. The stomach and duodenum were then injected with formol saline and examined after 24 hours fixation. The degree of gastric damage was recorded in each stomach after macroscopic examination by transmitted light with regard to the presence of necrotic areas in the mucosa, and to the number of haemorrhagic and non-haemorrhagic ulcers. The number of ulcers in each stomach was counted, and the mean number of ulcers per stomach in the group was calculated. Histological sections were stained with haematoxylin and eosin, with mucicarmine and PAS for mucin, and with Mann's methyl blue and eosin for examination of the peptic and parietal cells.

Microscopic examination of mast cells: Mast cells were examined in the tissues of rats subjected to irradiation in the experiments described in Chapter 4.

The skin for subcutaneous tissue spreads was obtained from the right side of the middle line of the abdomen. The skin

was separated from the under-lying tissue in the same way as it was for the samples which were assayed for histamine. The subcutaneous tissue was then removed from the deep surface of the skin and mounted by the method described by Riley (1953). The spreads were stained with 0.5% toluidine blue.

The first 2 cm of the jejunum and the anterior wall of the pyloric portion of the stomach were used for preparing paraffin sections. Half of each sample of tissue was fixed in formol saline and the other half in absolute alcohol. The tissue fixed in formol saline was stained with haematoxylin and eosin and that fixed in absolute alcohol was stained with toluidine blue.

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The methods for extraction and assay of histamine on the guinea-pig ileum have been used by numerous investigators. The question as to whether the histamine measured in this way is present in the circulating plasma in a physiologically-active state has been discussed by Dawlin (1957). He has shown that histamine in the guinea-pig ileum is

Most of the methods employed in the present experiments have been used frequently in the past by other investigators. Some of the procedures have, however, been developed for use in the present experiments. Amongst these is the simultaneous measurement of histamine by biological and radio-active assay in order to investigate the distribution and fate of histamine in intact animals. The biological assay of histamine in the blood and tissues measures histamine in a chemically unmodified form. Assay of the radio-active  $Cl_4$  measures the concentration of only a portion of the histamine molecule which is present in the tissues. This portion may still be in a biologically active form or may have been modified so that it is no longer biologically-active. In order to compare the concentrations of biologically-active histamine and radio-active  $Cl_4$  in the tissues, the latter has been translated into molecules of chemically complete histamine bihydrochloride and is referred to as "radio-active histamine," although there is no evidence that it was biologically-active, and indeed, it is clear that it was not biologically-active in many of the experiments. Under these conditions, two assays of the tissue histamine were made simultaneously; one measured the biologically active histamine which had been introduced into the tissues from exogenous sources, and the other measured the theoretical value of the total exogenous histamine which remained in the tissues. The values for the exogenous biologically active histamine and for the total exogenous radio-active histamine are expressed together in the results in Chapter 3. The method of exclusion of the rat kidneys from the circulation was also developed for use in these

experiments in order to investigate the part which the renal tissues played in controlling the distribution and fate of histamine in the living animal.

A method for the removal of the gastric secretion from the unanaesthetized intact guinea-pig at any desired time was developed in order to examine the secretion during the period when drug-induced changes were occurring in it, and while pathological changes were occurring in the gastric mucosa. Owing to the small diameter of the guinea-pig oesophagus, even in large animals, it is much more difficult to pass a stomach tube in this animal than in the rat, but, provided that certain details, such as keeping the animal's neck fully extended, are carefully observed during the procedure, it can easily be carried out and the full volume of gastric secretion can invariably be removed from the stomach (Watt & Wilson, 1958). These procedures have been used to obtain the results described in the following chapters, and their advantages will be described more fully in the appropriate sections.

The methods for extraction and assay of histamine on the guinea-pig ileum have been used by numerous investigators. The question as to whether the histamine measured in this way is present in the circulating plasma in a physiologically-active state has been discussed by Emmelin (1945). He has shown that histamine assayed biologically on the guinea-pig ileum is present in a physiologically active state in the plasma and tissues. This conclusion is of considerable importance in the present experiments where the metabolism of histamine is being examined by comparison of the tissue values of radio-active and biologically-active histamine, and observations on the concentrations of biologically-active histamine in pathological tissues have been made in order to investigate the part which histamine plays in producing these lesions. In the present experiments, it is assumed that biologically-active histamine is, or is capable of becoming very rapidly and easily, physiologically-active. The term "biologically-active" is used to describe such histamine when referring to the actual quantities or concentrations of it found in the tissues. The term "physiologically-active" is used when referring to histamine more generally as a substance having pharmacological effects on tissues in living animals.



### CHAPTER 3

#### THE METABOLISM OF EXOGENOUS AND ENDOGENOUS HISTAMINE

It is universally accepted that histamine, whether it be of exogenous or endogenous origin, is disposed of by the two processes of enzymic destruction by various oxidising enzymes, and excretion through the renal tract after it has been released into the circulating blood of the animal organism. By his experiments in which he used radio-active histamine and histidine, and then measured the excretion of their metabolic products, Schayer (1952a, 1952b) has shown that histamine can be formed in vivo by the injection of histidine, and that histamine itself, and at least two of its metabolic products, appear in the urine. There is, however, little information about the way in which histamine comes in contact with its specific enzymes, or about the way in which it is transferred throughout the body. The distribution of exogenous histamine has been investigated by Rose & Browne (1938) in the rat, and by Emmelin (1950) in the cat. Dale (1948) discussed the mode of action and distribution of endogenous histamine in the body and, in order to provide an explanation of why it could sometimes be antagonised by anti-histamines, and at other times anti-histamines were ineffective, he defined the terms intrinsic and extrinsic histamine. The histamine is called intrinsic when it is released by the cells which give the response, and extrinsic when it is released by one cell and acts upon another. Earlier, Feldberg (1937) had suggested that histamine liberated by the tissues goes into the tissues spaces and exerts its action for some time; it is presumably metabolized either there, or is transferred to the site where Schayer's enzymes can metabolise it, before it is finally excreted. The very rapid clearance of histamine from the blood after its intravenous injection (Rose & Browne, 1938), however, suggests that the rate of uptake of histamine by the tissues is of importance in controlling the activity of the metabolizing enzymes and in regulating the rate of its transfer in the kidneys. This aspect of the control of the rate of histamine metabolism may be of importance in the turnover both of exogenous and endogenous histamine, and forms the subject of investigation in the following experiments.

The distribution, fate, and turnover of histamine after its intravenous injection has been investigated by using radio-active histamine. The concentration of the radio-activity has been compared with the concentration of biologically-active histamine in the blood and tissues. The biologically-active histamine normally present in the tissues was measured in a large number of control animals (Table 3), so that the quantity of biologically-active exogenous histamine taken up by the tissues could subsequently be estimated. By observing the changes in the values for the two forms of histamine, the extent of the metabolism of exogenous histamine

TABLE 3

was calculated in the different experiments. The experiments were performed on normal animals and also on animals whose kidneys were excluded from the circulation by ligation of the renal pedicles. Content of biologically-active histamine in different tissues of the normal rat. Expressed in  $\mu\text{g/g}$  of histamine bihydrochloride.

Liver	1.6	1.3	2.5	2.7	2.7
Lungs	3.3	13.0	15.0	8.2	11.8
Muscle	9.7	2.4	7.3	12.9	18.4
Liver	3.0	1.2	1.4	1.4	2.6
Lungs	5.1	10.4	13.9	21.0	7.0
Muscle	7.3	18.5	12.0	11.0	16.8

Radio-active histamine was given by intravenous injection as described on page 4. Non-isotopic histamine acid phosphate was given to the depleted animals by intraperitoneal injection or by stomach tube by the methods described on page 3 and in Table 2.

The methods used for the extraction, and the biological and radio-active assays of histamine are described on pages 10 and 11. The experimental procedure for performing the nephrectomies, for depleting the skin of histamine, and measuring its urinary excretion are described on pages 6 and 8.

#### THE DISTRIBUTION OF ENDOGENOUS HISTAMINE IN NORMAL ANIMALS.

Blood histamine: The histamine content of the blood was measured by biological assay and measurement of its radio-activity after the intravenous injection of 5  $\mu\text{g/kg}$  of histamine  $\text{H}^3$ -HCl. The assays were generally performed on the plasma histamine only, but in some of the samples, changes in the cell histamine were also measured.

Changes in the concentration of plasma histamine are shown in fig. 1 and 1a. It can be seen that histamine leaves the circulating blood very rapidly after the intravenous injection. During the first minute, the concentration of both radio-active and biologically-active histamine fell rapidly. Subsequently, however, their rates of disappearance became slower, and, depending on whether the biologically-active or radio-active



was calculated in the different organs. The experiments were performed on normal animals and also on animals whose kidneys were excluded from the circulation by ligation of the renal pedicles as described on page 6. In this way, the processes of destructive oxidation and excretion of histamine by the kidneys were eliminated, and these effects on the turnover of histamine in the rat could be assessed.

The metabolism of endogenous histamine is of importance in conditions in which histamine has been released from the cells and is exerting physiological or pathological effects either intrinsically or extrinsically (Dale, 1948). In such circumstances, it might be expected that exogenous histamine could replace the histamine in the depleted tissues, and that this would affect the intensity and duration of its action in the animal. The ability of exogenous histamine to replace endogenous histamine in this way has been investigated by comparing the concentrations of biologically-active histamine in depleted skin in animals which had received injections of exogenous histamine, and control animals which had not received any histamine. Since the urinary excretion of histamine reflects the fate of histamine in the body (Gaddum 1951), it was also measured in depleted animals which had received histamine.

Radio-active histamine was given by intravenous injection as described on page 4. Non-isotopic histamine acid phosphate was given to the depleted animals by intra-peritoneal injection or by stomach tube by the methods described on page 8 and in Table 2.

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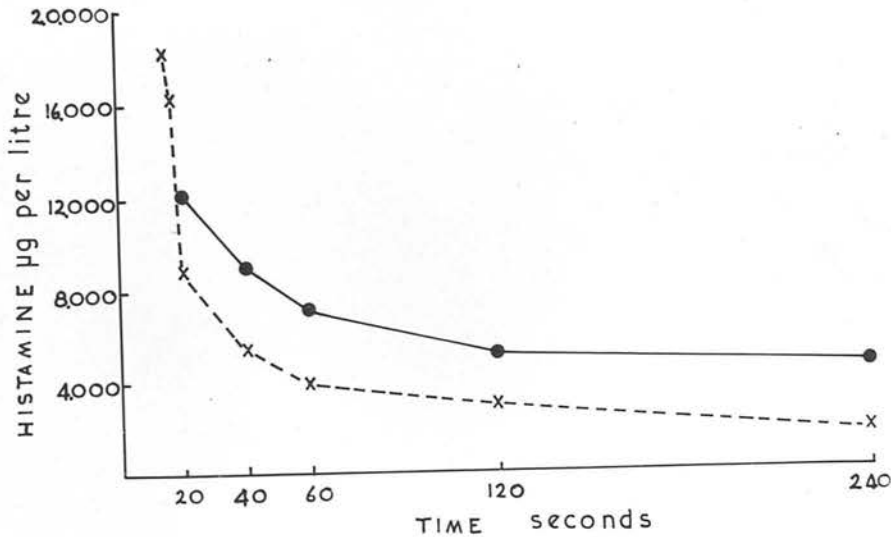


Fig. 1. Changes in the concentration of radio-active and biologically-active histamine in the plasma of the normal rat during the first 4 minutes after the intravenous injection of 5 mg/kg of histamine Cl<sub>4</sub> bihydrochloride. Radio-active histamine is calculated from the values for radio-active Cl<sub>4</sub>, and is expressed like the biologically-active histamine as µg/litre histamine bihydrochloride. Radio-active histamine ●—●  
Biologically-active histamine X--X

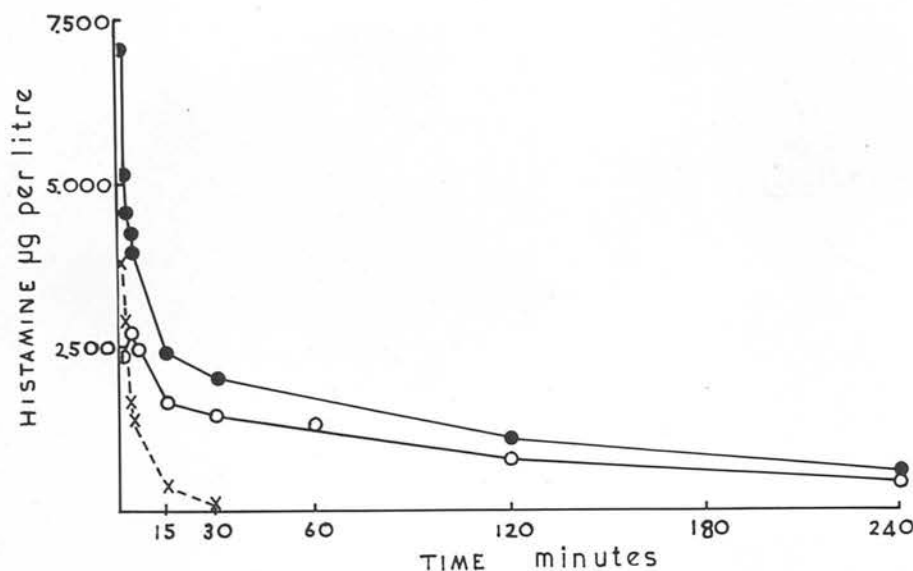


Fig. 1a. Changes in the concentration of radio-active and biologically active histamine in the plasma and blood cells of the normal rat during 4 hours after the injection of histamine  $Cl_4$  bihydrochloride. Radio-active histamine; in the plasma ●—●, in the cells ○--○. Biologically-active histamine in the plasma X--X.

The concentration of radio-active histamine in the cells was generally less than that in the plasma, but at the

the circulation of histamine from the circulating blood to the cells, but their results have been criticised on account of the large doses of histamine which they used. Such doses may cause disturbances in the circulation after intravenous injection. The varied values for the tissue histamine found in the present experiments following the injection of a relatively small dose of histamine confirm the observations of Rose & Brown (1958) and show that such effects occur in the absence of any circulatory changes induced by the histamine.

form was being examined, the fall continued for about 15 minutes or several hours.

The biologically-active histamine disappeared very rapidly from the plasma. Immediately after injection, when it had attained complete mixing with the extracellular fluid of which the volume is about 20 ml. in rats weighing 100 g, it would have a concentration of 25.000  $\mu\text{g/litre}$ . Fifteen seconds after its injection, the plasma only contained 18.300  $\mu\text{g/litre}$  and after one minute, it had fallen to 3.810  $\mu\text{g/litre}$ ; that is, about 85% of the biologically-active histamine had disappeared. The value had returned to normal 30 minutes after the injection. The concentration of radio-active histamine diminished more slowly than that of the biologically-active form. This difference had appeared during the first minute but it became more pronounced thereafter, and 30 minutes after the injection, the plasma still contained 2.010  $\mu\text{g/litre}$  or 8% of the theoretical concentration, at zero time. It was not until 4 hours after the injection that the concentration of radio-active histamine had almost disappeared.

The difference between the values for the radio-active and biologically-active histamine corresponds to the quantity of histamine which contains the inactive imidazole nucleus in the biologically-active preparation. By calculating the fraction biologically-active histamine

radio-active histamine  $\text{Cl}_4$ , the quantities of the active and inactive forms remaining in the circulation can be estimated, and the rate of metabolism of histamine can be measured. This has been done in Table 4 and the rate of metabolism of the biologically-active histamine as expressed by its decline from unity is shown in column 6.

Exogenous histamine penetrated rapidly into the cellular fraction of the blood and the fluctuations in its values resembled those in the plasma (Fig. 1a).

The concentration of radio-active histamine in the cells was generally less than that in the plasma, but at the end of 4 hours the values for the exogenous histamine in the cells and plasma had diminished to almost the same low level.

Tissue histamine: The rapid disappearance of histamine from the circulating blood suggested that a powerful mechanism existed for the uptake of histamine into the tissues. Rose & Browne (1938) reported a similar rapid disappearance of histamine from the circulating blood of the rat, but their results have been criticized on account of the large doses of histamine which they used. Such doses may cause disturbances in the circulation after intravenous injection. The varied values for the tissue histamine found in the present experiments following the injection of a relatively small dose of histamine confirm the observations of Rose & Browne (1938) and show that such effects occur in the absence of any circulatory changes induced by the histamine.

TABLE 4

Changes in the values for the radio-active and biologically-active histamine in the plasma and for the fraction Biologically-active histamine

Radio-active histamine in normal rats after the intravenous injection of histamine bi-HCl 5mg/kg. Injection at time zero.

Time after Injection	Biologically-active histamine No. of Animals    µg per litre		Histamine calculated from plasma CL4 No. of Animals    µg per litre		Biologically-active histamine <u>Radio-active histamine</u>
15 seconds	9	18.300	-	-	-
20	9	8.870	3	12.080	0.735
40	9	5.410	3	8.900	0.605
1 minute	9	3.810	9	7.050	0.540
2	7	2.910	9	5.150	0.550
3	-	-	6	4.560	-
4	7	1.685	6	3.770	0.450
5	28	1.404	6	3.470	0.400
15	21	0.360	3	2.420	0.150
30	21	0.100	3	2.010	0.047
60	-	-	-	-	-
120	-	-	3	1.080	-
180	-	-	-	-	-
240	-	-	3	0.560	-

The figures in Table 5 show that the kidney has an extremely active pump effect in comparison with the other tissues.



The rapid disappearance of histamine from the circulation suggested that the tissues were absorbing it, for the process appeared to be too fast wholly to be explained by its enzymatic destruction or urinary excretion. The rate of histamine uptake by various tissues was accordingly measured in terms of the content of radio-activity, and of their increase in concentration of biologically-active histamine. Biological assay was only performed on the tissues in which it was thought that the uptake or metabolism of the histamine would be greatest. On account of the high normal histamine content of the skin, the contribution made to its histamine content by the injection of exogenous histamine was relatively small, and insufficiently accurate to provide figures of any value.

Radio-active histamine rapidly diffused into the different tissues but the quantities in these tissues varied considerably. Its distribution in the blood and tissues at different intervals after the injection of 5 mg/kg is shown in fig. 2. The organs which contained the most histamine were the kidneys, striated muscle, liver, skin and ileum; elsewhere, the concentrations were considerably less. The uptake of biologically-active histamine was small in comparison with the quantity of the radio-active form which was found in the tissues. Its uptake by the kidneys was 57%, and by the striated muscle and liver was 41%, of the total, as indicated by the uptake of the radio-active form.

The quantity of biologically-active histamine in the tissues diminished rapidly. This decrease occurred as a result of two phenomena which took place simultaneously, the enzymatic destruction and renal excretion of the biologically-active form. The quantities of both forms of histamine found in the different tissues, at various periods, after its administration are shown in fig. 2. It can be seen that the proportion of radio-active histamine increased as a function of time, and that as the quantity of histamine diminished in the tissues, it increased in the urine.

On account of the different weights of the tissues, it is of value to calculate the histamine uptake not in terms of the whole organ, but per gram of the tissue. This makes it possible to find out whether any of the tissues take it up in a preferential fashion.

The histamine uptake by the different tissues is shown in Table 5. It can be seen that 5 minutes after the injection of radio-active histamine, the kidney has absorbed the largest quantity of histamine, namely 146  $\mu\text{g/g}$ . The heart, liver and lung follow in descending order, each having absorbed about one-tenth as much as the kidney, and the striated muscle contained the lowest concentration. The uptake of biologically-active histamine in terms of its concentration in the tissues resembled its uptake by the entire organs.

The figures in Table 5 show that the kidney has an extremely active pump effect in comparison with the other tissues.

TABLE 1

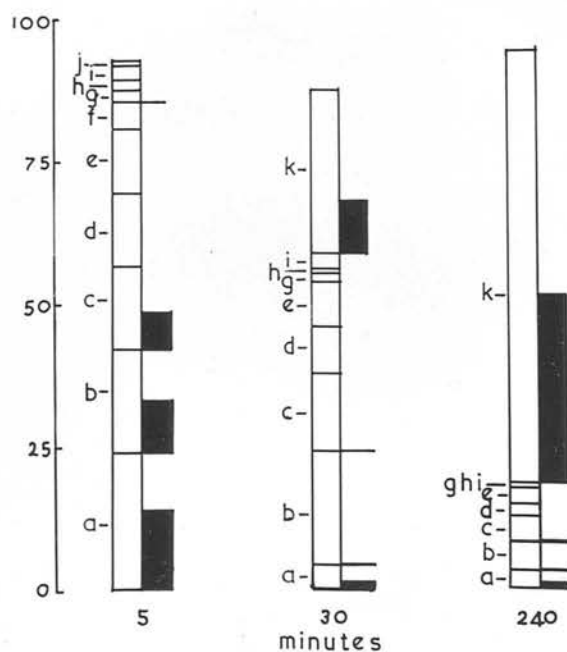


Fig. 2. Percentages of radio-active and biologically-active histamine found in the blood, urine, and different organs after the intravenous injection of Cl<sub>4</sub> histamine bihydrochloride. The figures express the values per whole organ. The skin, striated muscle, and blood have been taken as 15, 45 and 8% of the body weight respectively. a, kidney; b, striated muscle (thigh); c, liver; d, abdominal skin; e, ileum (without contents), f, whole blood; g, lungs; h, myocardium; i, colon (without contents); j, spleen; k, urine. Radio-active histamine; white columns. Biologically-active histamine; black columns.

TABLE 5

Content of histamine CL<sub>4</sub> in the organs. Values represent the mean of 3 animals and are expressed in  $\mu\text{g/g}$  of the fresh organ.

Tissue	5 minutes	30 minutes	240 minutes
Kidney	146.5	23.9	15.3
Heart	21.0	8.6	1.3
Liver	16.9	17.1	5.4
Lung	10.6	7.8	1.4
Ileum	9.8	6.7	3.5
Colon	5.6	5.1	1.6
Spleen	5.5	4.8	2.3
Stomach	5.4	2.1	1.9
Skin	4.2	2.9	0.7
Muscle	2.0	2.4	0.6

**Tissue Histamine:** The levels of the biologically and radio-active histamine in the tissues are shown in Fig. 1 at various intervals after the intravenous injection of histamine. It is clear that the radio-active histamine was distributed throughout all the remaining organs when the kidneys were excluded from the circulation, and was not selectively concentrated in any of them. Most of the tissues took up some of the injected histamine; the uptake varied between 2 and 3% of the total, according to the organ. The most interesting observation made in these experiments on nephrectomized animals was that a transfer of radio-active histamine took place between the organs. Certain tissues, such as the liver, ileum and heart, obviously absorbed the histamine initially at the expense of the other tissues. Later, they lost this store of histamine which passed to the muscle and skin. The quantity of biologically-active histamine stored in the organs decreased progressively following the

This powerful extracting ability of the kidney has also been observed recently by Lindell & Schayer (1958) in the dog. The predominating effect of the kidney in controlling the transfer of histamine in the living animal made it desirable to study its metabolism in animals whose kidneys had been excluded from the circulation in order to investigate more fully the metabolic effects of the remaining organs.

#### THE DISTRIBUTION OF EXOGENOUS HISTAMINE IN NEPHRECTOMIZED ANIMALS.

By excluding the kidney from the circulation, it was possible both to prevent the kidney from taking part in the absorption of the histamine, and to eliminate the role of the kidney as an organ for the excretion of histamine.

Blood histamine: The values for the radio-active and biologically active histamine in the plasma in nephrectomized animals following its intravenous injection are shown in Fig. 3. and Table 6. The rate of disappearance of the histamine from the circulation during the first minutes was similar in its general appearance to that in normal animals. However, the values for the histamine remained about 30% higher in the nephrectomized animals. Although the values for the biologically-active histamine were not obtained during the first minute, it is clear that the values were considerably raised in the animals whose kidneys had been excluded from the circulation and that its rate of disappearance was reduced in comparison with normal animals. The concentration of radio-active histamine in the plasma diminished during the first 30 minutes after the histamine had been injected, but thereafter, it did not change significantly for a period of several hours. As was the case in normal animals the changes in the concentration of radio-active histamine in the blood cells were similar to those in the plasma although the concentration in the cells remained about 2% less during the whole experiment.

Tissue Histamine: The levels of the biologically and radio-active histamine in the tissues are shown in fig. 4 at various intervals after the intravenous injection of histamine. It is clear that the radio-active histamine was distributed throughout all the remaining organs when the kidneys were excluded from the circulation, and was not selectively concentrated in any of them. Most of the tissues took up some of the injected histamine; the uptake varied between 2 and 5% of the total, according to the organ. The most interesting observation made in these experiments on nephrectomized animals was that a transfer of radio-active histamine took place between the organs. Certain tissues, such as the liver, ileum and heart, obviously absorbed the histamine initially at the expense of the other tissues. Later, they lost this store of histamine which passed to the muscles and skin. The quantity of biologically-active histamine stored in the organs decreased progressively following the



TABLE 4

Changes in the values for the radio-active and biologically-active histamine in the plasma, and for the fraction Biologically-active histamine in nephrectomized rats after the intravenous injection of histamine  $Cl_4$  HI-HCl 5 mg/kg.

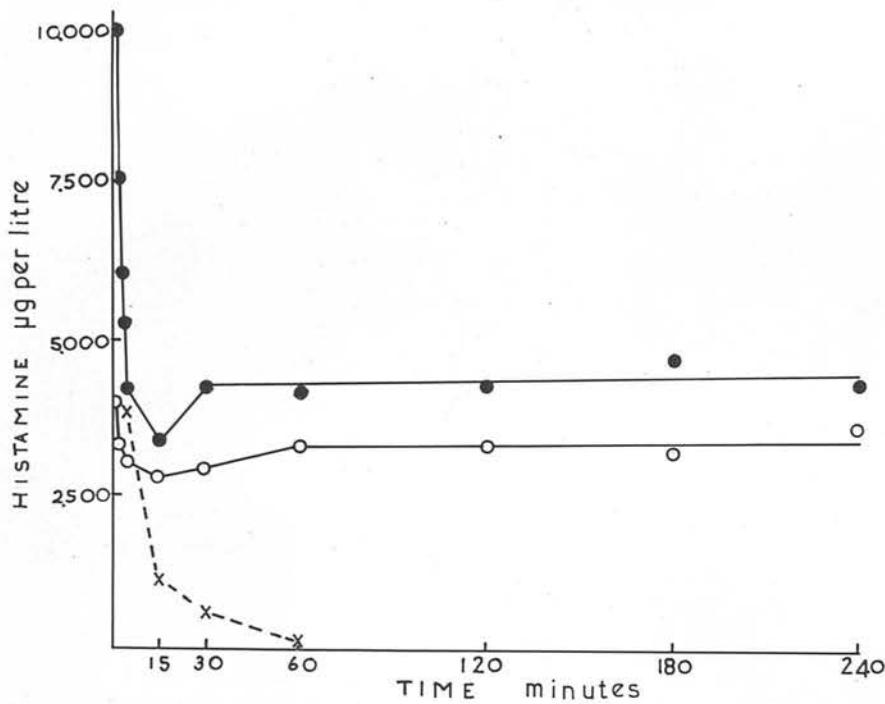


Fig. 3. Changes in the concentration of radio-active and biologically-active histamine in the plasma and blood cells of the rat with kidneys excluded from the circulation during 4 hours after the injection of histamine  $Cl_4$  bihydrochloride. Radio-active histamine; in the plasma ●—●; in the cells ○--○. Biologically-active histamine in the plasma X--X.

TABLE 6

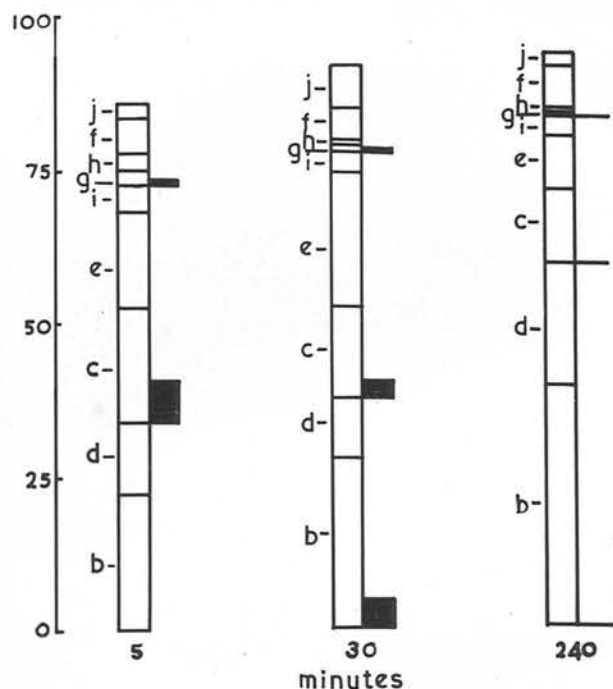
Changes in the values for the radio-active and biologically-active histamine in the plasma, and for the fraction Biologically-active histamine

Radio-active histamine in nephrectomized rats after the intravenous injection of histamine  $Cl_4$  BI-HCl 5 mg/kg. Injection at time zero.

Time after injection Minutes	Biologically-active histamine		Histamine calculated from plasma $Cl_4$		Biologically-active histamine
	No. of Animals	$\mu$ g per litre	No. of Animals	$\mu$ g per litre	<u>Radio-active histamine</u>
1	10	10.000	6	10.110	0.99
2	10	6.615	6	7.586	0.88
3	10	4.775	3	6.066	0.80
4	10	4.685	3	5.250	0.89
5	30	3.924	4	4.240	0.92
15	20	1.100	3	3.360	0.33
30	20	0.617	3	4.160	0.15
60	20	0.145	3	4.140	0.04
120	20	-	3	4.240	-
180	20	-	3	4.700	-
240	-	-	3	4.260	-

injection; and the fraction biologically-active histamine correspondingly diminished. This indicates that a progressive destruction in the

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metabolism of histamine by the different organs was found to be a very rapid process as can be seen from Table 6. When the metabolic rate was measured per gram of tissue, the kidney appeared to have considerable metabolic activity in comparison with the other organs. However, no definite

**Fig. 4.** Percentage of radio-active and of biologically-active histamine found in the blood and different organs after the intravenous injection of Cl<sub>4</sub> histamine bihydrochloride. For explanation of the letters, see legend to Fig. 2.

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any particular organ. It is  
clear that the cellular destruction of histamine in the rat is a  
very rapid process. Exclusion of the kidneys from the  
circulation did not alter this rate of degradation of  
biologically-active histamine by the tissues as can be seen  
from Table 7.

The excretion of both forms of histamine in the urine was measured during a period of 4 hours after the injection of histamine. After 30 minutes, 27%, and after 240 minutes, 70% of the injected histamine had been excreted in the form of radio-

injection; and the fraction biologically-active histamine  
radio-active histamine  
correspondingly diminished. This indicates that a progressive  
destruction of the biologically-active histamine was occurring  
in the tissues.

Turnover of exogenous histamine in the tissues: After the  
administration of histamine marked with  $^{14}\text{C}$  in the imidazole  
nucleus, information is obtained about the movement and fate  
of the radio-active histamine without knowing whether the  
molecule is still biologically-active or has been broken down.  
Biological assay alone indicates whether the injected histamine  
is present in the blood and tissues in an unaltered form. The  
percentage of the injected histamine which has been metabolised  
at any instant in the tissues is indicated by the percentage of  
the radio-active histamine which is biologically inactive. This  
can be calculated from the following formula:

$$100 - \frac{(\text{Total biologically-active histamine found} - \text{biologically-active histamine normally present}) \times 100}{\text{Radio-active histamine}} \\ = \% \text{ Radio-active histamine metabolized.}$$

Figures which show the rate at which the radio-active  
histamine in the plasma was metabolized are given in Table 7.  
These figures indicate that the biologically-active histamine  
disappeared rapidly, and that about 5 minutes after its injection,  
60% of the radio-active histamine had been transformed into a  
physiologically inactive form. In the nephrectomised animals,  
the rate of transformation was apparently reduced although a  
larger number of observations will be necessary definitely to  
confirm this.

Metabolism of histamine by the different organs was  
found to be a very rapid process as can be seen from Table 8.  
When the metabolic rate was measured per gram of tissue, the  
kidney appeared to have considerable metabolic activity in  
comparison with the other organs. However, no definite  
conclusions can be drawn from this observation, on account of  
the dynamic state of the turnover and metabolism of histamine by  
the tissues. This enabled histamine and its metabolites to be  
transported from one organ to another without definite proof that  
the histamine was katabolised in any particular organ. It is  
clear that the cellular destruction of histamine in the rat is a  
very rapid process. Exclusion of the kidneys from the  
circulation did not alter this rate of degradation of  
biologically-active histamine by the tissues as can be seen  
from Table 7.

The excretion of both forms of histamine in the urine  
was measured during a period of 4 hours after the injection of  
histamine. After 30 minutes, 27%, and after 240 minutes, 76%  
of the injected histamine had been excreted in the form of radio-



TABLE 7

Fall in the values of the biologically-active histamine calculated as a percentage of the radio-active histamine in the plasma of normal and nephrectomized rats after the injection of 5 mg/kg of histamine Cl<sub>4</sub> bi-HCl at zero time.

Time - Minutes	Normal Animals	Nephrectomized Animals
1	37	1
2	36	12
3	-	20
4	56	11
5	60	8
15	88	73
30	95	85
60	-	97

TABLE 8

The rate of metabolism of biologically-active histamine in the different tissues. Time in minutes: Values expressed as per centage of biologically-active histamine metabolized.

Time	Control Rats			Nephrectomized Rats		
	5	30	240	5	30	240
Kidney	43	72	82	-	-	-
Liver	59	100	-	42	81	100
Lung	73	100	100	77	66	83
Striated Muscle	45	100	100	-	86	100

active CL4 (Fig. 2). The quantity of biologically-active histamine recovered in the urine, varied between 14 and 45% of the total histamine which was injected. From tables 4 and 7, it can be seen that the destruction of the biologically-active histamine was almost complete after 4 hours. It would, therefore, be expected that the renal excretion of the biologically-active histamine and its metabolites would be almost complete also at the end of this period. The figures for the recovery of biologically-active histamine and its inactive metabolites in the urine confirm this, and are in close agreement with those obtained by Schayer (1952b) in the rat, who recovered 95% of the injected radio-active histamine 20 hours after its injection, of which 11% was in the form of pure histamine.

#### THE RESTORATION OF ENDOGENOUS HISTAMINE IN DEPLETED ANIMALS.

The fact that exogenous histamine is rapidly taken up by the tissues of normal rats makes it desirable to determine whether exogenous histamine can also be absorbed by tissues depleted of their endogenous histamine. This problem has been investigated by measuring the ability of the depleted skin to absorb intravenously administered histamine, and examining the urinary excretion of histamine in order to find out whether exogenous histamine is affected by the general metabolic processes of the body after histamine depletion.

It is well known that the skin of the rat cannot be depleted completely of its histamine, but owing to the extensive depletion which can be produced by compound 48/80, in this tissue, the skin was chosen to determine whether the administration of histamine would accelerate the rate of reformation of the normal histamine content. Gaddum (1951) has shown that estimation of free histamine in the urine is the most sensitive way of detecting liberation of histamine in the body. Comparison of the quantity of histamine excreted in the urine after its administration to rats before and after they have received compound 48/80, therefore, can give a fairly sensitive indication of whether there has been any uptake of histamine by the depleted tissues.

The experiments were carried out by measuring the normal values for the concentrations of biologically-active histamine in the skin or urine and then depleting the animals of histamine by the parenteral administration of compound 48/80. The histamine was given by injection or by mouth 2 - 3 days after the last injection of compound 48/80, and its uptake measured. The urinary excretion of histamine was measured over a period of a few hours after the rats had been given a water-load.

The effect of exogenous histamine on the concentration of histamine in the depleted skin: In these experiments, the rats were depleted of their skin histamine by a course of injections of compound 48/80 which lasted for 4 days as described in

Chapter 2. On the fifth and sixth days, one group received histamine by injection or through a stomach-tube, or histidine by injection, and the control group received saline. The doses of histamine and histidine which were administered and the results of these experiments are summarized in Table 9.

It can be seen that the administration of histamine did not influence the rate at which it was reformed in the depleted skin. The dose which was injected was considerably in excess of that used in the previous experiments; this dose should, therefore, have been sufficient to cause a significant uptake of histamine by the skin, if this had been possible in depleted animals. The gross depletion of the skin would have allowed any change in the concentration of histamine to be easily recognised, and thus the difficulty of measuring the uptake of histamine by the skin in normal rats, which was pointed out earlier, (page 22), would not apply in the present experiments. Administration of histamine by stomach-tube increases the excretion of conjugated-histamine (Wilson 1954b). It is possible that the changes produced in the histamine molecule during its absorption from the alimentary canal would enable it to be bound by the tissues. However, this did not occur (Table 9). These results confirm those of Schayer & Smiley (1954) who also found that exogenous histamine was not bound by the skin in depleted rats. In normal rats, the uptake of radioactive histamine by the skin is very small, (Fig. 2 and Table 5), and it is possible that the break-up of mast cells and resulting disturbance of histamine metabolism in this tissue caused by Compound 48/80 would reduce the uptake further.

Schayer (1952a) states that histidine is probably the only source of bound histamine in the tissues. The administration of histidine to depleted rats did not increase the histamine content of the skin, so that if histidine is the source from which endogenous histamine is formed in these circumstances, the increase in concentration which it produces in the skin is too small to be detected by biological assay.

The urinary excretion of exogenous histamine in depleted animals:

The urinary excretion of histamine was measured in normal animals after the administration of oral and intraperitoneal doses of histamine. The excretion was measured after the rats had received a water-load, and the histamine excreted in excess of normal as a result of administration of the histamine was determined as described on page 8 and Table 2. The rats were then depleted by injecting Compound 48/80 during four days, as described on page 5, and the excretion of histamine was measured again on the sixth day. The histamine was administered by intraperitoneal injection in three experiments and by stomach-tube in two experiments; the results are summarized in Tables 10 and 11.



TABLE 9

The effect of administration of histamine or histidine on the skin histamine content of rats depleted of their skin histamine by compound 48/80. Skin histamine:  $\mu\text{g/g}$  expressed as histamine base.

Normal Rats	Dose of Histamine or Histidine	Depleted Rats which received Saline	Histamine or Histidine
35.7	Histamine	10.0	11.2
30.6	12.5 mg/kg/day	16.8	10.9
31.6	1 P 1	11.3	9.8
<u>24.6</u>		<u>9.2</u>	<u>9.0</u>
30.6	Mean	11.8	10.2
28.5	Histamine	5.3	8.1
40.0	37.5 mg/kg/day	4.4	7.4
32.3	Oral	3.6	5.0
<u>48.5</u>		<u>7.4</u>	<u>5.4</u>
37.4	Mean	5.2	6.5
30.1	Histamine	16.1	6.6
37.7	112.5 mg/kg/day	10.9	6.3
25.1	Oral	18.2	10.3
<u>37.0</u>		<u>10.7</u>	<u>7.2</u>
32.5	Mean	13.9	7.4
34.3	Histidine I P 1	4.8	3.4
21.4	125 mg/kg/day	6.9	6.7

TABLE 10

Urinary excretion of histamine during control period before administration of histamine in normal rats and in the same rats after the injection of compound 48/80. Histamine values expressed as ng. base excreted per half hour.

Experiment	Histamine excretion		Last Injection Compound 48/80
	Normal	Depleted	
1	481	1491	3 days previously
2	568	683	2 do.
3	1349	2808	2 do.
4	682	894	2 do.
5	603	1037	2 do.

TABLE 11

Urinary excretion of histamine following the administration of a histamine load to normal rats and subsequently to the same rats after treatment with Compound 48/80. Histamine values expressed as ng base.

Histamine dose $\mu\text{g/kg}$	Histamine excess excretion		% of dose excreted	
	Normal	Depleted	Normal	Depleted
150 IP1	4227	2752	14.09	9.17
150 IP1	4382	3854	14.61	12.85
150 IP1	2814	1979	9.38	6.60
1000 Oral	3061	1868	0.31	0.19
1000 Oral	2982	2012	0.30	0.20

The results in Table 10 show that there was a slight increase in the urinary excretion of histamine in the depleted rats. This was present although the last injection of compound 48/80 had been given to all the rats at least 48 hours, and to some of them, 72 hours previously. It is possible that some of the histamine released by the last injection of compound 48/80 was still being excreted, but this is unlikely because it was shown by Wilson (1954a) that all the histamine released by a single injection of compound 48/80 in normal rats has been excreted within  $2\frac{1}{2}$  hours. It would not be expected that there would be a large or prolonged excretion of histamine resulting from the action of the liberator in rats which had already received increasing doses of compound 48/80 so that its histamine-releasing action was practically exhausted. Marshall (1958) has reported that cortisone causes the release of histamine from the alimentary canal and other tissues, and has suggested that stress itself may be responsible for this effect. Compound 48/80, as a result of the effects produced by the massive release of histamine at the beginning of the experiment, may have stimulated the adrenal cortex and thus caused the release of cortisone. The increased excretion of histamine would then arise as a result of a stress effect and would persist for a few days after the last injection of the liberator.

There was a significant reduction in the quantity of the histamine load excreted by the rats after they had been depleted by compound 48/80. This occurred after both intraperitoneal and oral administration of the load. Between 3% and 5% less of the load was excreted by the depleted rats, so that even though these animals were excreting more histamine in their urine, they absorbed a larger quantity of the physiologically-active exogenous histamine. Since only the biologically-active form was being measured, it cannot be decided from these results whether this histamine was actually absorbed and stored by the tissues, or was metabolised in them and then excreted in the inactive form. The percentages of the dose excreted by the rats when they were normal, and when they were depleted, bore the same ratio whether the histamine was given parenterally or orally. It is clear, therefore, that oral administration did not affect the ability of the tissues to take up the histamine.

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Histamine diffuses very rapidly through the circulating blood volume after its intravenous injection. If the figures for the concentration of histamine in the plasma are used to calculate the volume of extracellular fluid in which the histamine is distributed one minute after its injection, it is found to have a diffusion space of about 70 ml per 100 g in the rat, which is greater than the total volume of water contained in the whole animal. This observation led to the conclusion that histamine must diffuse out of the extracellular fluid into the cells where it is stored in concentrations higher than those found in the blood itself. Emmelin (1950) made similar observations after injecting histamine into the cat, and also concluded on the basis of his calculations that the histamine passed in large quantities into the tissues where it was subsequently inactivated.

These observations do not agree with Schayer's statement (1956a) who says, with reference to exogenous histamine in the rat, "I do not believe that the exogenous histamine is picked up by the tissues". It has been shown in the present experiments that radio-active histamine is absorbed by all the tissues in the rat with the exception of the central nervous system. However, it is very likely that the exogenous histamine is deposited in the cells in a biochemical form which is different from the endogenous form. In none of these animals, in spite of many trials, was it possible to observe the reappearance of the radio-active histamine in the circulation after the administration of a histamine releaser. Schayer & Smiley (1954) have shown that such a release does occur when radio-active histamine is formed in the cells from histidine. The quantity of exogenous histamine which was absorbed varied from one tissue to another, but the absorption of both radio-active and biologically-active histamine was greatest in the kidney. This confirms the findings of Rose & Browne (1938) who also found that the absorption of biologically-active histamine was greatest in the kidney of the rat.

Study of the distribution of histamine and its metabolites in the normal animal is affected by their rapid excretion by the kidney. This makes it difficult to study the metabolism and transfer of histamine through the tissues. Exclusion of the kidneys from the circulation overcame this difficulty, and the two-stage character of the operation prevented the undesirable effects produced by complete bilateral nephrectomy on the day of the experiment. In the nephrectomized animals, the biologically-active histamine decreased progressively while the radio-activity persisted in the blood at a constant level from 15 minutes until several hours after the injection. The biologically-active histamine was being progressively metabolised, and a diffusion equilibrium between the histamine and its inactive metabolites became established across the cell membranes. During this period also, a transfer of radio-active  $Cl_4$  from the liver and splanchnic viscera took place towards the striated muscle and lungs.

The most interesting information obtained from these experiments concerns the metabolism of histamine in the different tissues. The techniques which were employed offered particularly favourable conditions for this kind of investigation. However, before any conclusions can be drawn regarding the metabolic activity of the organs by comparing the values of the two forms

of histamine, the fact that histamine and its metabolites are transported all the time from one to another of the organs must be taken into account, and thus the metabolites found at any time in the tissues may not originally have been produced there. The present results show that the metabolism of histamine by the tissues is extremely rapid, almost 100% of the histamine taken up by the tissues being metabolised in 30 minutes. It is clear that exogenous histamine in normal animals can be metabolised in large quantities and insofar as this conclusion can be applied to particular tissues, this metabolic process appears to occur especially in the case of the kidney.

These experiments which were made in nephrectomized animals enabled the tissue metabolism of histamine to be examined without the necessity of making any correction for the urinary excretion of histamine. However, the kidney possesses considerable ability to excrete histamine. As has been shown, it can excrete 14 - 15% of an injected dose of histamine in the unchanged form. It is practically the only excretory organ for histamine since only negligible quantities of the injected histamine, in the region of 1%, have been found in the lumen of the stomach or intestine.

It has been shown that endogenous histamine can be released in large quantities from the tissues in a variety of conditions. Under severe conditions, this is associated with degeneration of the mast cells and the production of oedema, fall of blood pressure and the occurrence of damage in the gastrointestinal tract. It has been pointed out by Emmelin (1945) that the presence of physiologically-active histamine in the blood may produce different degrees of physiological activity not only in animals of different species, but also in animals of the same species under different conditions. Thus, the relationship between physiological action and blood concentration of histamine is not yet fully defined and may clearly be influenced by the very rapid absorption and metabolism of histamine by the tissues. In normal animals which have received histamine intravenously, the duration of its physiological effect will be limited on account of the rapidity with which it is metabolized and excreted.

The intensity and duration of the action of endogenous histamine will similarly depend upon its speed and mode of release. Provided that the metabolic and excretory activities of the animal are undisturbed, the released endogenous histamine will be dealt with as rapidly as it can be disposed of from the blood and extra cellular fluid. As has been shown, however, such disposal depends upon the uptake and metabolism of histamine by the tissues. Schayer (1956b) states that only histidine can be bound by mast cells; the increased proportion of the histamine load absorbed by the depleted rats must, therefore, have been taken up by other tissues. Since the mast cells had all been destroyed there must also have been a more rapid turnover of histamine by the other tissues to account for the increased urinary excretion of histamine in the depleted animals. It can be concluded that

the ability of animals whose mast cells have been destroyed, to absorb and excrete physiologically-active histamine is not impaired, and indeed, that the rate of turnover of histamine by the tissues may actually be increased.

Irradiation produces a characteristic sequence of macroscopic and microscopic changes in the tissues of all animals. It has been shown that changes occur in the blood histamine following irradiation (Weber & Stegenga, 1949) and it has been suggested that histamine may play a part in producing the physiological and pathological changes which follow. It is clear that endogenous histamine may be involved in the changes which follow irradiation of living tissues in at least two ways;

(i) There may be a disturbance in the metabolism of histamine in the irradiated areas.

(ii) Histamine itself may play a part in causing the tissue destruction which is produced by irradiation.

Although, for purposes of investigation, it is convenient to divide the metabolic and pathological action of histamine in this way, it is possible that they are actually dependent, and that a disturbance in the metabolism may produce changes in the tissues of the irradiated animals.

There have been few previous investigations on these aspects of the metabolism and action of histamine, and so it was considered that it would be of value to investigate them, especially since any changes found in the metabolism of endogenous histamine as a result of irradiation, might be related to, or explainable in terms of, the metabolism of histamine in the normal animal which was described in the last chapter. Prosser (1947) has investigated the general metabolic changes which follow irradiation and he attempted to correlate them with the clinical features of the radiation syndrome. Peisberg & Langer (1954) were the first to show that there was a reduction in the skin histamine in man, although the observations were made in only two cases. Experimental evidence about the tissue histamine content is scanty, although it has been suggested that histamine liberated from irradiated cells or possibly newly formed from irradiated histidine in the tissues may be responsible for some of the effects of radiation sickness (Allinger, 1951). Weber & Stegenga (1949) have shown that there is an increase in blood histamine levels in the rat after irradiation and Vanders & Peisberg (1955) state that dogs and rabbits became more sensitive to infusion of histamine. It is therefore apparent that some changes occur in the metabolism of histamine after irradiation, but they have not been investigated, or related to the clinical features of the radiation syndrome, to the same extent as this has been done with the morphological changes which accompany the syndrome.

CHAPTER 4

THE ROLE OF ENDOGENOUS HISTAMINE IN RADIATION DAMAGE  
OF THE TISSUES

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Changes in the histological appearance of the tissues have been widely investigated and Bloom (1947) has attempted to correlate these changes with the clinical features following irradiation. Bloom (1948) has also investigated the macroscopic and microscopic changes in the skin which follow  $\beta$ -irradiation. Oedema and vasodilatation occur early and are followed by degenerative changes in the cells and vascular occlusion; erythema and ulceration occur in the living tissue. Ungar & Damgaard (1954b) have studied the effect of  $\beta$ -radiation on the histamine content of the skin of rats and on the vascular response of the skin to injections of compound 48/80 in the irradiated areas. There have, however, been no other attempts to relate the macroscopic and microscopic changes in  $\beta$ -irradiated skin with its content of histamine.

In the present experiments, the effects of total and partial body irradiation have been investigated. The effects of various doses have been examined both on the tissue content of histamine and on the changes induced by radiation in the tissue. Rats were used as the experimental animals because of the bigger content of histamine in their tissues which made small changes easily discernable, and because it was known that they were susceptible to both X- and  $\beta$ -irradiation. The irradiation was administered to the rats by the methods described on pages 8 & 10.  $\beta$ -irradiation was used for high intensity radiation of the skin but there were no significant differences between the two types of radiation with regard to their histological effects on the skin. The histamine content of the skin and alimentary canal were measured by biological assay, and the changes which occurred have been correlated with the histological changes in these tissues, in particular, with the changes in the mast cells. Since both histamine and heparin are constituents of the mast cells, the coagulation time was also examined before and after irradiation. The effect of  $\beta$ -irradiation was also studied on the response of the blood-vessels to injections of histamine and compound 48/80. In order to determine the part which histamine plays in the production of irradiation lesions in the skin, the inflammatory and degenerative changes were investigated in rats which had previously been depleted of histamine by injections of compound 48/80, and these results have been compared with those in undepleted animals.

#### THE EFFECT OF TOTAL BODY IRRADIATION.

The dose of 1025 r total body irradiation which was administered to twelve rats caused the death of three of them after 15 days, and of five in 25 days. The animals lost weight during the week following irradiation, but in the animals which survived, this weight loss was subsequently regained.

#### THE EFFECT OF IRRADIATION ON THE SKIN.

##### The effect of X-irradiation on the skin.

Histamine content of the skin: Riley & West (1953) have shown

that the histamine content of various tissues, including the skin, is closely related to the numbers of mast cells which they contain. Depending therefore on the thickness of the skin, and the quantity of subcutaneous tissue which is removed with it, the histamine content of the skin will vary. In the present experiments, as far as possible, skin of the same thickness and skin from the same part of the abdomen was always used. In fig. 5 there is shown a section in order to demonstrate the thickness of the skin which was used and its content of mast cells. The mean value for the histamine content of the abdominal skin from eleven rats was  $32.6 \mu\text{g/ml}$  with a standard error of 1.3. The effect of 605 r on the histamine content of the abdominal skin is shown in fig. 6. Throughout the first 24 hours, and on the third day, after the irradiation, the histamine value remained within the normal range, but on the fifth post-irradiation day it fell, and reached the minimum value of  $18.9 \mu\text{g/ml}$  on the tenth day. Thereafter, it increased slightly. The observations at each time period were made on at least six rats.

In order to investigate the effect of doses of 1000 r on the abdominal skin histamine, the rats were partially protected by lead shields which diminished the mortality produced by the higher doses to the abdominal skin. Several days after irradiation of the abdominal skin its histamine content had fallen from the control value of  $30.5$  to  $12.6 \mu\text{g/g}$  and then fell slightly further on the tenth post-irradiation day to  $12.0 \mu\text{g/g}$ . The percentage effect of doses of 605 and 1000 r are shown in fig. 7, in which it can be seen that the larger dose produced a greater fall in the skin histamine value, although the effect produced by each dose occurred after the same latent period. A dose of 2000 r from a circular applicator produced no changes in the histamine content of the abdominal skin 20 hours after local irradiation in comparison with adjacent unirradiated skin. Seven days after irradiation with 2000 r, the skin histamine values in the irradiated areas in two rats were 7.8 and  $13.7 \mu\text{g/g}$ , whereas the histamine contents of adjacent unirradiated areas were 25.1 and  $30.1 \mu\text{g/g}$  respectively.

The mast cells of the subcutaneous tissue: The changes in the appearance of the mast cells after irradiation resembled those described by Riley & West (1955a) following the injection of 'subacute doses' of compound 48/80 intraperitoneally into rats, except that they occurred more gradually. Twenty-four hours after irradiation, the majority of the cells showed normal staining reactions and contained tightly packed granules. A few of the cells were swollen and the granules were less tightly packed than in normal cells. On the fifth post-irradiation day (fig. 8a), the mast cells were swollen and the cell membranes of many of them were ruptured. Granules had leaked out of the cells into the surrounding tissue; these granules appeared to be stained in the usual way. Other cells had a large ill-defined pale area in the centre and the granules were concentrated irregularly at one side of the cell. The smaller mast cells

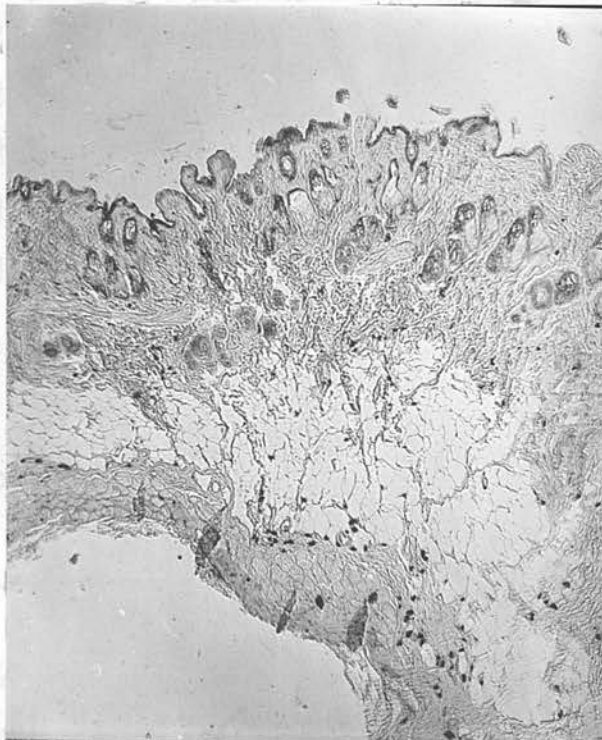


Fig. 5. Section of normal abdominal skin showing the thickness which was used for histamine estimations and the mast cell content. Toluidine Blue X 35.

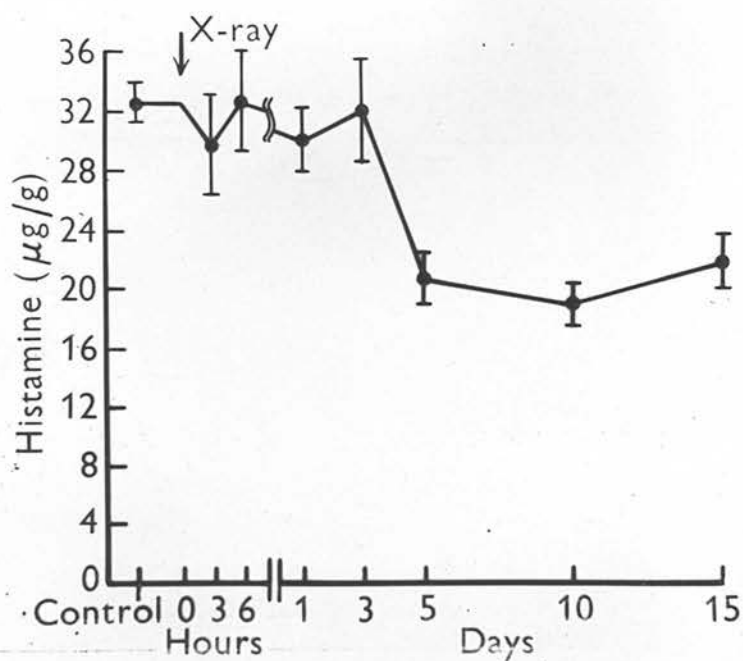


Fig. 6. Effect of 605 r X-irradiation on the histamine content of abdominal skin. The standard error of the observations at each time interval is shown.

Fig. 7. The changes in the histamine content of the abdominal skin, following different doses of X-irradiation applied to the skin, expressed as percentages of the normal values. 0-0.605 r; 0-0.1000 r.



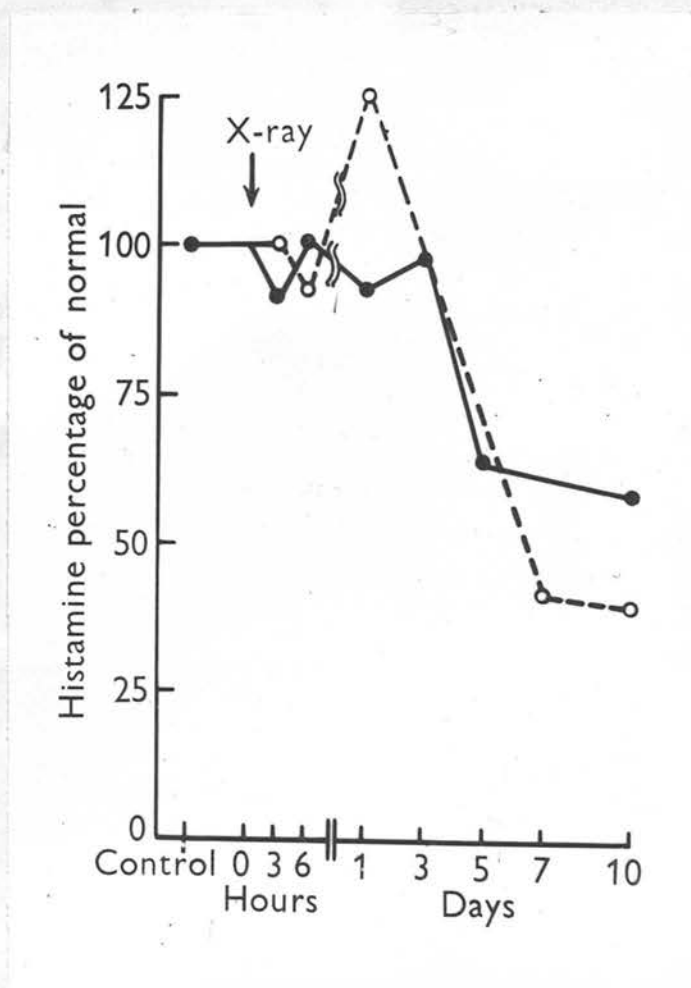
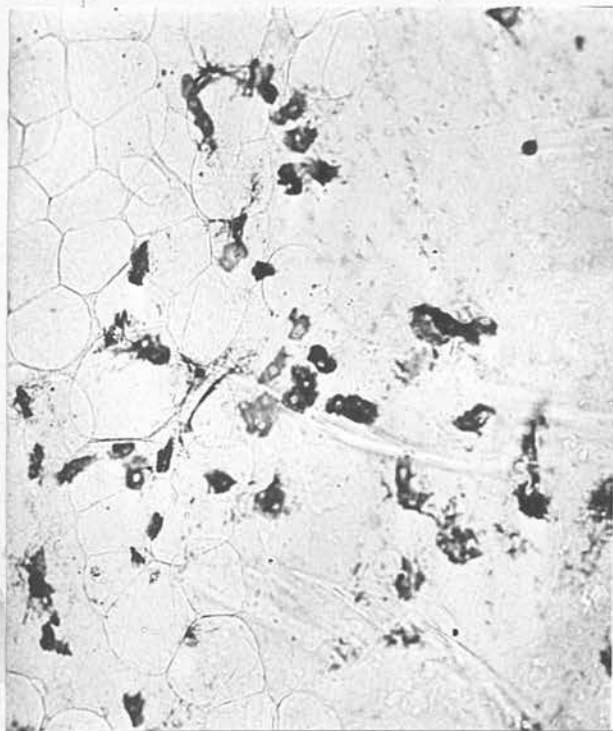
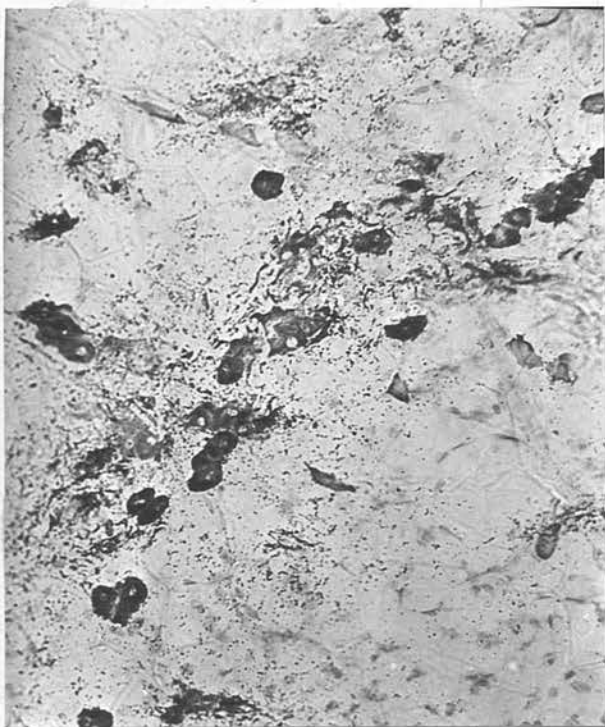


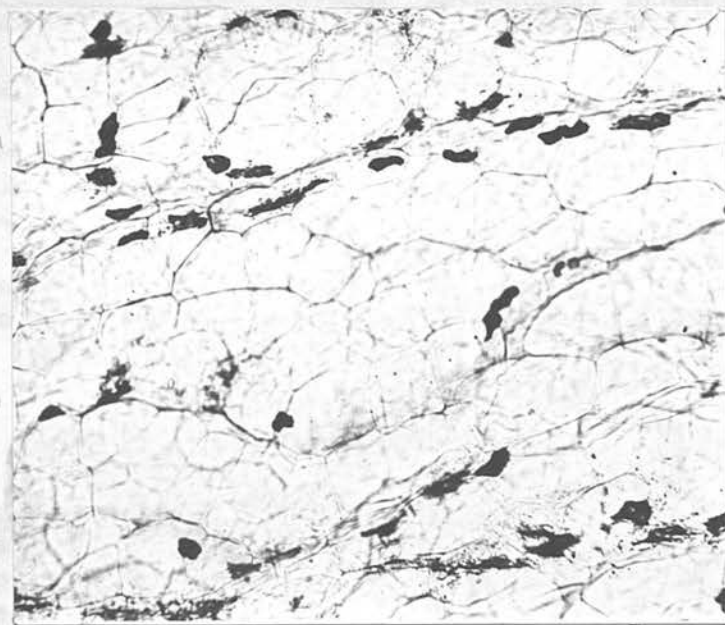
Fig. 7. The changes in the histamine content of the abdominal skin, following different doses of X-irradiation applied to the skin, expressed as percentages of the normal values. ●-●, 605 r; ○-○, 1000 r.



a



b



c

Fig. 8. Subcutaneous tissue spreads following 605 r X-irradiation to abdominal skin. Toluidene blue, X 190.

- a) Fifth day following irradiation. Swelling of the mast cells and rupture of the cell membranes with dispersion of granules in the adjacent tissue.
- b) Tenth day following irradiation. Swelling and extensive disintegration of the mast cells with wide dispersion of granules.
- c) Fifteenth day following irradiation. Small new cell formation along the capillary at the bottom of the photograph. Disintegration of cells and dispersion of granules still visible at the top of the photograph.

close to the blood vessels also had ruptured cell membranes. The total number of cells in the tissue had obviously diminished. The connective tissue cells showed increased basophilia and the connective tissue strands were visible as a basophilic network.

Ten days after irradiation, mast cells could be seen as very pale ghost cells or had entirely disappeared in localized areas. Elsewhere, the cell membranes had ruptured and the granules were scattered through the surrounding tissue (fig. 8b). A few small deeply stained cells were visible along the blood vessels. These cells represented the commencement of recovery of the tissues from the effects of irradiation by the development of new cells from the adventitia of the blood cells (Riley & West, 1955a). The connective tissue network was still deeply stained with the basic dye. On the fifteenth day (fig. 8c), the mast cells consisted of a mixture of deeply stained small cells along the vessels and larger deeply stained cells in the tissue remote from the vessels. A few cells with ruptured membranes and granules lying round them in the tissues, were still visible.

Local reactions of skin vessels: Intradermal injections of either histamine or compound 48/80, at all concentrations tested, elicited the same response in the normal skin and in the skin which had been irradiated locally 20 hours earlier with 2000 r. Thus at the time when the response of the skin vessels to histamine and compound 48/80 was examined, the histamine content of the skin had not decreased. Pontamine sky blue appeared in the normal and irradiated skin over approximately equal areas and with the same intensity (fig. 9). In many rats the colour seemed to develop earlier at the irradiated site, but this difference soon vanished and was not noticeable by the tenth minute. Intradermal injections of saline produced only traces of local blueing.

#### The Response of normal skin to $\beta$ -irradiation.

The macroscopic appearance of the skin: The dose of  $\beta$ -radiation to the skin was larger than that used with X-radiation. Macroscopic changes occurred in the skin which were observed until the tenth day following irradiation and resembled those described by Ungar and Damgaard (1954). Desquamation and erythema occurred on about the sixth day. A sticky exudate then appeared, sloughing of the skin surface occurred and a shallow ulcer had formed by the tenth post-irradiation day. The irradiated skin contracted so that the irradiated area was reduced to about half of its initial size by the tenth post-irradiation day. No hair growth occurred in the irradiated area (fig.10).

The histamine content of the skin: The histamine content of the skin from the non-irradiated control areas remained within the normal range of 32.6  $\mu\text{g/g}$  with a standard error of 1.3 during

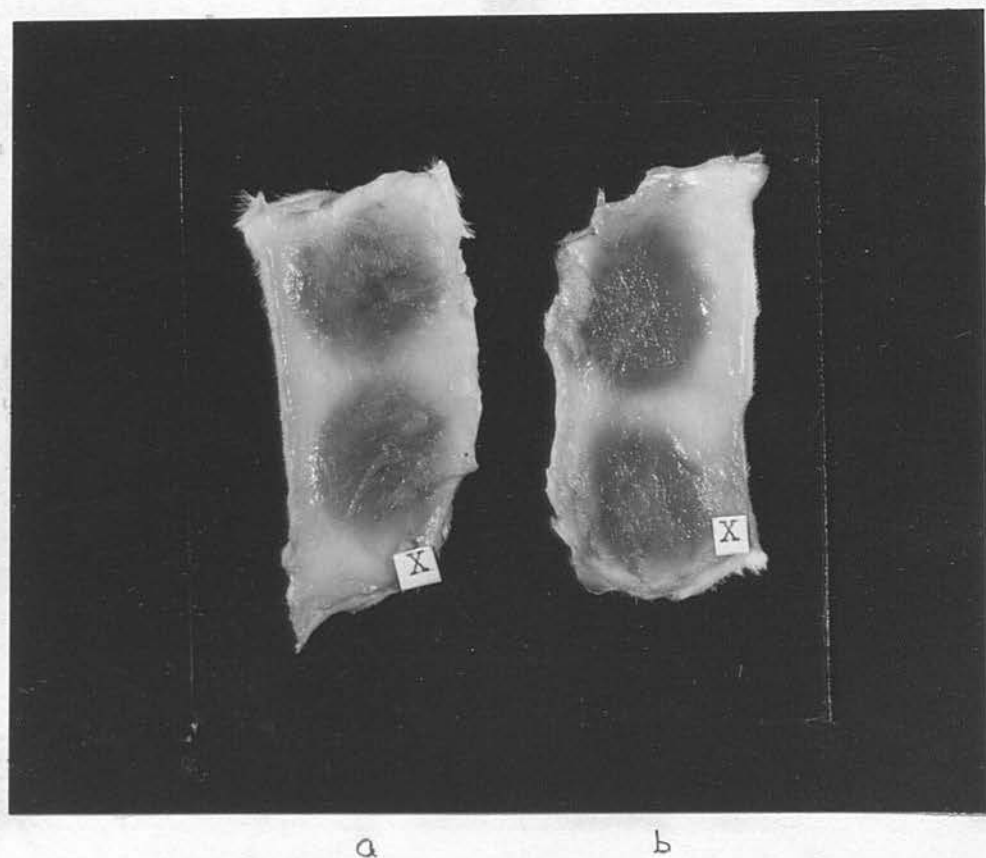


Fig. 9. Local skin reactions in X-irradiated, and adjacent normal abdominal skin. X=irradiated areas. Blueing in response to intradermal injections of

a) Compound 48/80 1/30,000

b) Histamine 1/20,000.



the ten days following  $\beta$ -irradiation. The histamine content of the irradiated areas decreased following irradiation. The decrease began after 24 hours but it did not become pronounced until the fifth day when the mean histamine content of the irradiated area had fallen to 14  $\mu\text{g/g}$  at which it remained until the tenth day (Table 12).  $\beta$ -irradiation therefore decreases



a

b

Fig. 10. The appearance of the ulcerated area in irradiated skin ten days after  $\beta$ -irradiation:

- a) in a normal rat
- b) in a histamine-depleted rat.

the ten days following  $\beta$ -irradiation. The histamine content of the irradiated areas decreased following irradiation. The decrease began after 20 hours but it did not become pronounced until the fifth day when the mean histamine content of the irradiated skin had fallen to 14  $\mu\text{g/g}$  at which it remained until the tenth day (Table 12).  $\beta$ -irradiation therefore caused a gradual decrease in the skin histamine concentration which fell to 43% of the value of the control samples of skin from the same rats (fig.11).

The mast cells of the subcutaneous tissue: The mast cells showed degenerative changes similar to those in the skin following X-irradiation. There were no significant changes in the cells eight hours after irradiation, but at twenty hours, some of the cells were swollen and the granules were less tightly packed than in normal cells. Three days after irradiation, some of the cells were swollen and in some the cell membrane was ruptured. On the fifth post-irradiation day, the cell membrane was broken in many of the cells; the granules had leaked into the surrounding tissues and were less deeply stained than normal. Most of the other cells were deformed and although some showed vacuolation, a few deeply staining cells could still be seen (fig.12). Ten days after irradiation, many of the cells had an ill-defined pale area in their centres and granules could not clearly be distinguished. Fragmented cells with their granules scattered through the surrounding tissue were still visible and some small deeply staining cells, indicative of new cell formation (Riley & West, 1955a), were also scattered in small groups through the tissue.

Local reactions of the skin vessels: Intradermal injections of histamine into normal and irradiated skin produced responses of the same size and intensity of blueing at both sites. These reactions occurred with all the histamine concentrations which were used. Intradermal injections of compound 48/80 also caused responses of the same size and intensity in normal and irradiated skin in all the concentrations which were tested, including that used by Ungar and Damgaard, (1954), (fig.13). Intradermal injections of saline produced only traces of local blueing. The responses of the irradiated skin to the injections of histamine or compound 48/80 were of the same character twenty hours after  $\beta$ -irradiation.

The blueing responses were tested in a few rats on the seventh post-irradiation day when the skin histamine had fallen to one-third of its normal value. Histamine and compound 48/80 in concentrations of 1/10,000 produced equal responses in normal and irradiated skin.

The effect of  $\beta$ -irradiation and depletion by Compound 48/80.

$\beta$ -irradiation followed by depletion with compound 48/80. In view of Ungar & Damgaard's report that the wheal-formation which is normally associated with intradermal injection of compound 48/80



TABLE 12

The histamine content of normal and  $\beta$ -irradiated abdominal skin.

Rat	Time after $\beta$ -irradiation	Skin histamine content $\mu\text{g/g}$			
		Normal	Mean	Irradiated	Mean
1	8 hours	31.4	31.4	27.7	27.7
2	20	29.4	29.8	24.4	23.5
3	20	30.2		22.6	
4	3 days	24.0	25.5	22.6	20.3
5	3	26.9		17.9	
6	5	30.2	29.1	11.1	14.0
7	5	27.9		16.8	
8	10	30.0	33.5	12.7	13.9
9	10	37.0		15.1	

Fig. 11. The changes in the histamine content of the abdominal skin following  $\beta$ -irradiation, expressed as percentages of the values for the histamine content in adjacent normal skin.  $\beta$ -irradiation at zero time.

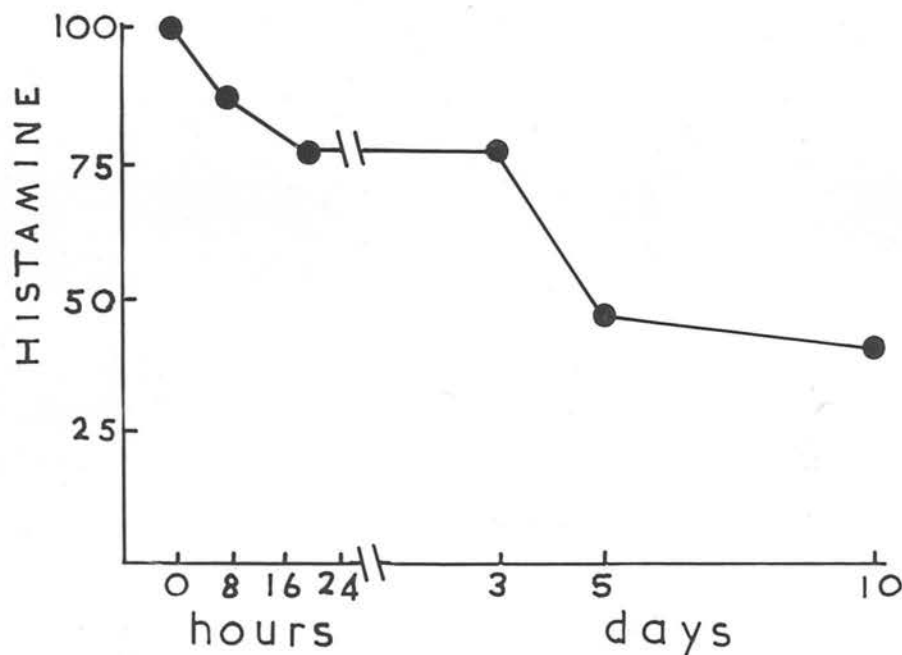
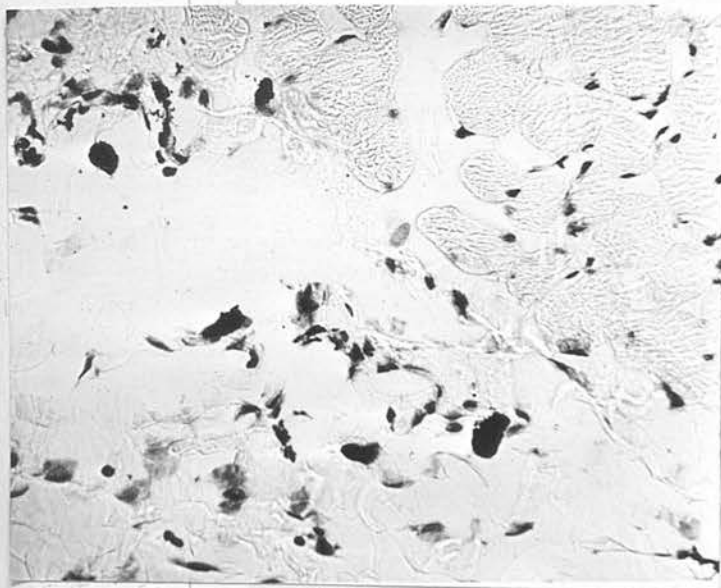
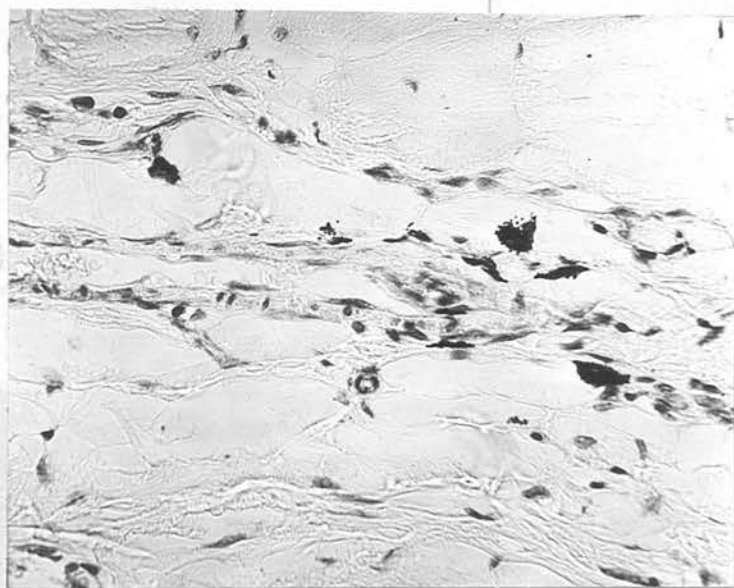


Fig. 11. The changes in the histamine content of the abdominal skin following  $\beta$ -irradiation, expressed as percentages of the values for the histamine content in adjacent normal skin.  $\beta$ -irradiation at zero time.

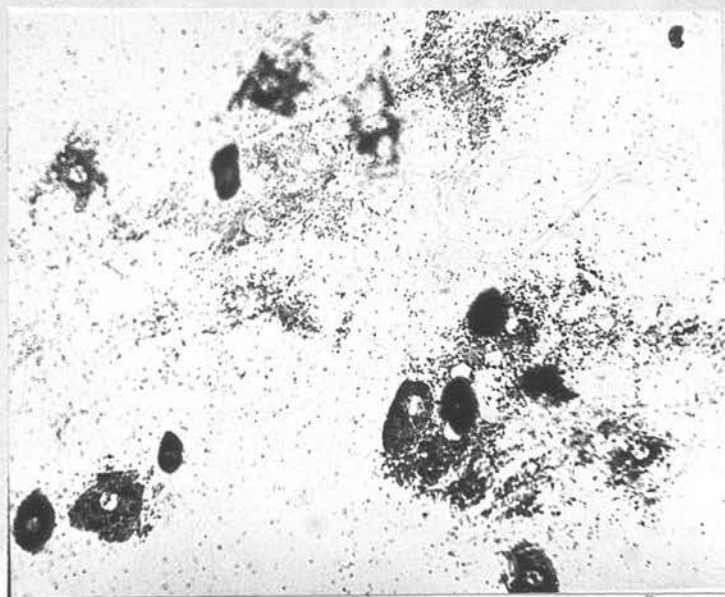




a



b



c

Fig.12. Mast cells in the abdominal skin following B-irradiation  
Toluidine blue X 460.

- a) Normal skin. Paraffin section.
- b) Fifth day following irradiation. Paraffin section.  
Deformation of cells, early vacuolation and rupture  
of cell membranes.
- c) Tenth day following irradiation. Subcutaneous  
tissue spread. Vacuolation and extensive  
disintegration of the mast cells with wide  
dispersion of granules.

be prevented throughout the period of skin healing up to nine days after exposure to radiation, an experiment was performed in order to determine whether  $\beta$ -irradiation would prevent the histamine liberating action of compound 48/80 in the skin during this period.

The rats were exposed to  $\beta$ -irradiation and on the following day, depilation with compound 48/80 was attempted. Skin was removed for estimation of the histamine content from depilated

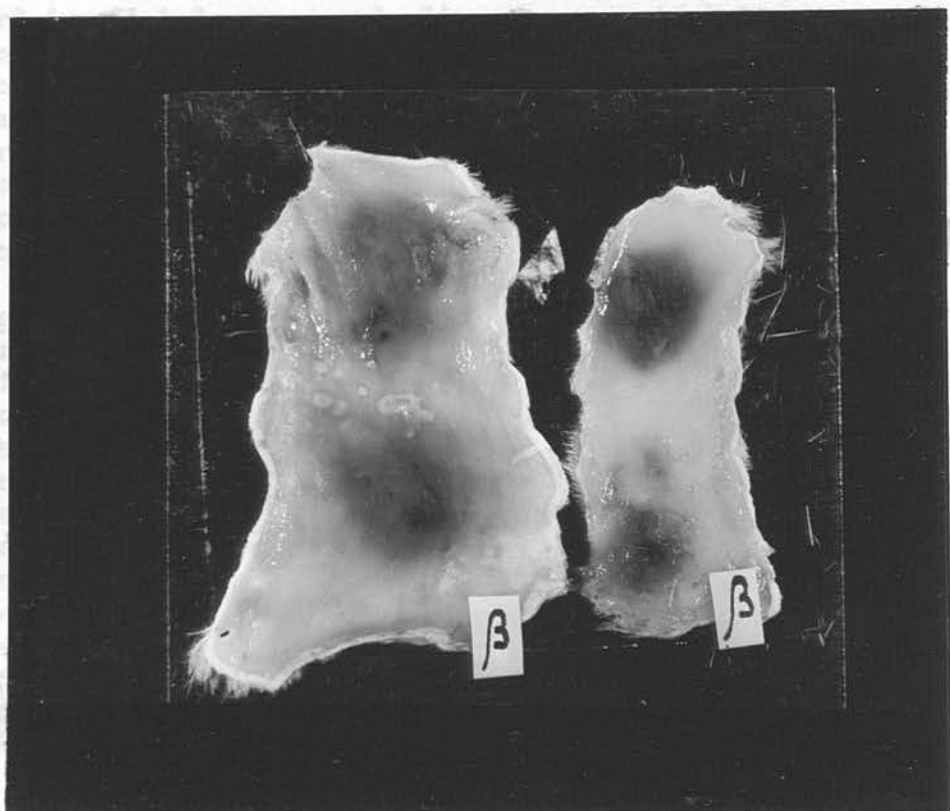


Fig. 13 Local skin reactions in  $\beta$ -irradiated and adjacent normal abdominal skin.  $\beta$  = irradiated area. Blueing in response to intradermal injections of:

- a) Compound 48/80 : 1/10,000
- b) Histamine : 1/2000

Histamine content of rat skin and plasma. The histamine content of the plasma, serum or the extract in skin was 23.4  $\mu\text{g/g}$  with a standard error of 1.2. It began to decrease during the first 24 hours after total body irradiation with 100% and reached the minimum value on the eighth day. Thereafter, it increased slightly. The skin still slightly

is prevented throughout the period of six hours, up to nine days, after exposure to radiation, an experiment was performed in order to determine whether  $\beta$ -irradiation would prevent the histamine liberating action of compound 48/80 in the skin during this period.

The rats were exposed to  $\beta$ -irradiation and on the following day, depletion with compound 48/80 was commenced. Skin was removed for estimation of the histamine content from depleted and irradiated areas, and adjacent areas which had been depleted only. In none of the rats was any difference in histamine content detected between the two areas of skin, as is shown in Table 13. From these results, it appears that previous  $\beta$ -irradiation does not diminish the histamine-liberating effect of compound 48/80 and that compound 48/80 and  $\beta$ -irradiation together, do not diminish the histamine content of the skin more than compound 48/80 alone.

Depletion with compound 48/80 followed by  $\beta$ -irradiation: Several experiments were performed in which various degrees of depletion with 48/80 were produced, and were followed by  $\beta$ -irradiation in order to determine whether the histamine depletion would affect the severity of the subsequent radiation lesions. The method of treatment and skin histamine values are shown in Table 14. In none of the rats was the size or progress of the radiation lesions different from the lesions in rats which had not received compound 48/80 (fig.10). In undepleted and in histamine depleted rats slight variations had appeared in the regularity of the margins of the ulcerated areas and in the degree of cicatrization of the ulcers by the tenth day after irradiation. These differences were not consistent and occurred in both groups irrespectively.

It has been reported that on the second day after the commencement of depletion with "subacute doses" of compound 48/80, a decrease in skin histamine, and mast cell destruction, have begun to appear. On the third day, mast cell destruction is complete (Riley & West, 1955a), and the skin histamine reaches a value which is less than that produced in the present experiments by  $\beta$ -irradiation alone. From the present experiments it appears therefore, that the severity of the subsequent ulceration is not modified by the level of skin histamine at which radiation was performed. It is also of interest that the rate of restoration of histamine in the area of skin which had been subjected to irradiation and depletion was similar to that in skin which had been depleted alone.

#### THE EFFECT OF IRRADIATION ON THE ALIMENTARY CANAL.

Histamine content of the stomach and jejunum: The histamine content of the pyloric portion of the stomach in eight rats was 23.4  $\mu\text{g/g}$  with a standard error of 1.7. It began to decrease during the first 24 hours after total body irradiation with 1025 r and reached its minimum value on the fifth day (fig.14). Thereafter, it increased slightly but it was still significantly

TABLE 13

Skin histamine content in rats subjected to  $\beta$ -radiation followed by depletion with compound 48/80.

Rat	Skin histamine content $\mu\text{g/g}$		Day of Death
	$\beta$ -radiation and depletion	Depletion only	
1	10.0	11.3	3
2	12.4	15.7	3
3	11.0	9.7	3
4	9.5	10.0	3
1	3.0	2.4	4
2	2.4	4.2	4
1	2.5	2.9	5
2	2.6	2.7	5
1	2.9	2.8	6

$\beta$ -radiation given on day 1; injections of compound 48/80 commenced on day 2.



TABLE 14

Skin histamine content in rats subjected to depletion by compound 48/80 followed by  $\beta$ -radiation

No. of Rats	Compound 48/80	Day of $\beta$ -radiation	Day of death	Mean histamine content of skin	
				Irradiated and depleted	Depleted only
2	Daily for 4 days	2	5	3.0	2.7
3	Daily for 6 days	2	7	4.4	3.7
1	Daily for 10 days	2	18	10.8	12.7
3	Daily for 15 days	5	16	3.5	3.6

Injections of compound 48/80 commenced on day 1.

Fig. 14. Effect of 1025 r total body  $\beta$ -irradiation applied to the skin of the back on the histamine content of the stomach and jejunum. The standard error of the observations at each time interval is shown.

below the control value 15 days after irradiation. The observations at each time period were made on three rats.

The mean value for the histamine content of the jejunum obtained from the control rats was  $19.5 \mu\text{g/g}$  with a standard error of 1.5. Three hours after irradiation, the histamine content had already begun to diminish, by the end of 24 hours, it had fallen almost to its minimum value. On the tenth day the

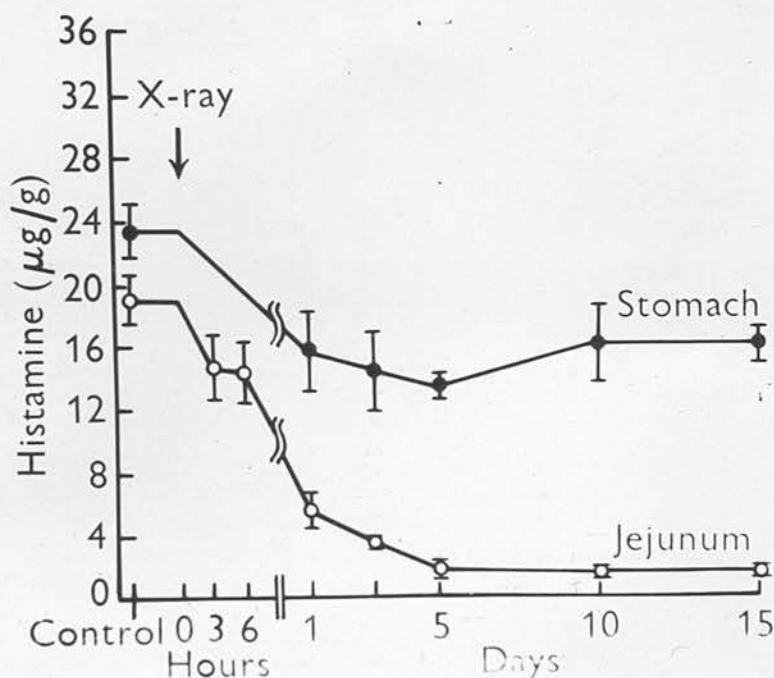


Fig.14. Effect of 1025 r total body X-irradiation applied to the skin of the back on the histamine content of the stomach and jejunum. The standard error of the observations at each time interval is shown.

below the control value 15 days after irradiation. The observations at each time period were made on three rats.

The mean value for the histamine content of the jejunum obtained from the control rats was  $19.0 \mu\text{g/g}$  with a standard error of 1.5. Three hours after irradiation the histamine content had already begun to diminish, by the end of 24 hours, it had fallen almost to its minimum value. On the tenth day the histamine content had fallen to  $1.4 \mu\text{g/g}$  at which it remained (fig.14).

The changes in the histamine contents of the different tissues following total body irradiation have been compared in fig.15. Whereas the histamine content of the stomach wall and skin decreased to about 60%, that of the jejunum fell to 7.5% of the normal value. The figure also illustrates that the changes in the skin occur considerably later than those in the stomach and jejunum.

The epithelium of the stomach and jejunum: The cells in the wall of the stomach passed through the sequence of changes which normally follow irradiation (Pierce, 1948). At the end of 24 hours, the mucous cells forming the gastric pits and the necks of the mucous glands were poorly stained and the nuclei were pyknotic. The granules in the peptic cells were pale and amorphous and in most of the parietal cells there was vacuolization of the cytoplasm and the nuclei had disappeared or were pyknotic. Mitoses appeared in the cells during the following days and by the fifth post-irradiation day, the mucous neck cells were reappearing and there were many fresh cells in the bases of the gastric glands. Numerous mitoses were present in the peptic cells and their granules had reappeared. The parietal cells had resumed their normal appearance.

Twenty-four hours after irradiation there was degeneration of the columnar cells in the crypts of the jejunum, particularly in their superficial portions. There was pallor of the cytoplasm and many nuclei showed pyknosis. The goblet cells showed vacuolization. The lamina propria was oedematous and many of the cells were shrunken. On the third and fifth post-irradiation days, there was progressive growth of the deeply staining columnar cells from the bottom of the crypts. The goblet cells at the bases of the glands were stained normally and contained granules. The columnar epithelium at the surface was degenerating and sloughing off into the lumen of the canal. On the tenth day, deeply staining columnar cells extended more than half of the distance from the bases of the glands to the surface, and there were numerous goblet cells amongst them. Cells of Paneth had reappeared at the bases of the glands. The lamina propria was still slightly oedematous and pyknotic nuclei were visible in it but it was beginning to resume its normal appearance.

The mast cells of the stomach and jejunum. The mast cells in the submucosa of the stomach showed a degree of swelling similar to those in the submucosal tissue and vascular endothelial cells. Twenty-four hours after irradiation with 1025 r the stomach was still normal. In some there was complete preservation of the granules at one side, but none of the rest. However, the ruptured mast cells had no granules in the region of nuclei (Fig. 16a). On the third post-irradiation day there was a 20%

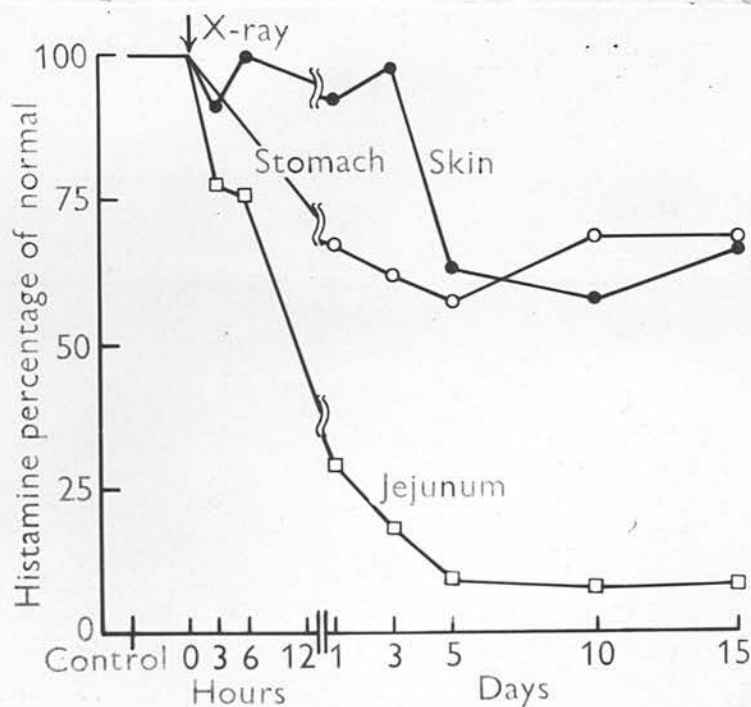


Fig. 15. The changes in the histamine content of the abdominal skin, the stomach, and the jejunum of the rat following 1025 r total body X-irradiation, expressed as percentages of the normal values.



The mast cells of the stomach and jejunum: The mast cells in the submucosa of the stomach showed a series of changes similar to those in the subcutaneous tissue and occurring at similar times. Twenty-four hours after irradiation most of the cells were still normal. In some there was central pallor with concentration of the granules at one side, but none of the cell membranes was ruptured and there was no decrease in the number of cells (fig. 16a). On the third post-irradiation day there were a few ruptured cell membranes and a few granules had leaked into the surrounding tissue. On the fifth post-irradiation day there was general rupturing of the cell membranes and granules were scattered through the surrounding tissue. Many of the cells showed vacuolation. On the tenth day (fig. 16b), after irradiation, the appearance of the cells was similar although a few deeply stained cells could now be seen beside the blood vessels. Very few mast cells could be seen in the wall of the jejunum of the normal rat. The cells became more difficult to find after irradiation, but in those which were seen, it appeared that a sequence of changes occurred in them similar to that seen in the gastric wall.

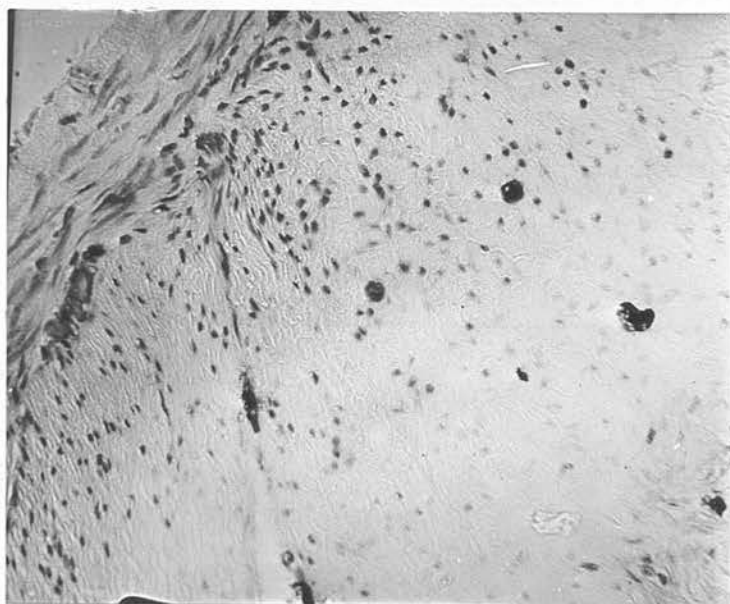
#### THE EFFECTS OF IRRADIATION ON LIVING TISSUES.

The blood coagulation time: No gross increase in the coagulation time occurred during the 15 days following total body irradiation with 1025 r, and the day-to-day variation which did occur was not significant (fig. 17). This agrees with the observations of Cohn (1952) who found no change in the coagulation time in female rats following irradiation with 400 r.

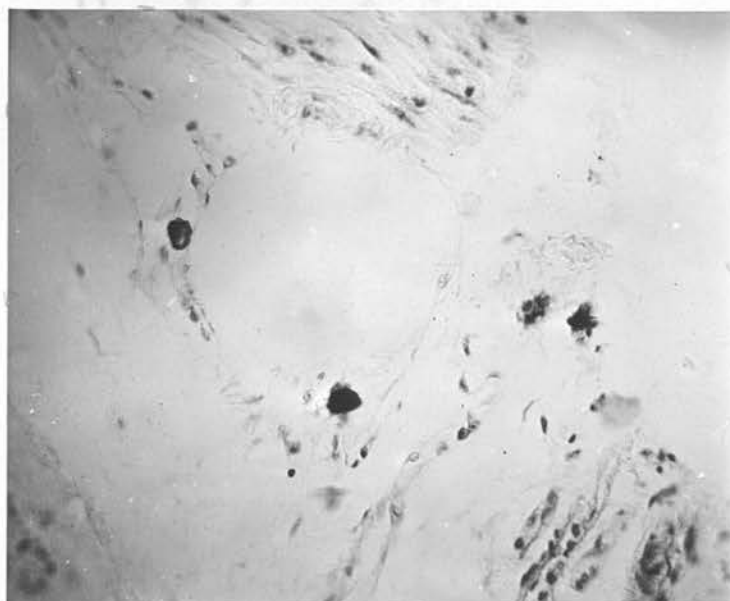
Comparison of the different effects produced by irradiation: Irradiation of the skin from either X or  $\beta$  sources caused a depletion of the skin histamine, which appeared after 3 days and gradually increased to its maximum 5 - 10 days after irradiation. The character of the histamine loss from the skin was the same after both types of irradiation, but its intensity varied according to the dose which was used (Table 15). It is interesting to observe that the histamine depletion reached a minimum value of 39% with a dose of 1000 r, and that increasing the dose had no further effect on the histamine concentration in the skin, although it is well-known that histamine liberators will reduce the skin content of histamine to 10% of its normal value.

A comparison of the various effects produced by body irradiation with 1025 r is given in Table 16, which also includes results obtained by Cohn (1952) on the heparin coagulation time after body irradiation with 400 r. The effects on the histamine value of the skin, on the mast cells, and on the heparin coagulation time appeared at the same time after irradiation and reached their maximum during the following days. The effects on the histamine content of the alimentary canal, and on the epithelium lining the canal, appeared during the first twenty-four hours, but were maximal several days before the histamine fell to its minimum value.

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a



b

Fig. 16. Section of the submucosa of the stomach following 1025 r total body X-irradiation. Toluidene blue x 190.

- a) Twenty four hours after irradiation. No significant changes in the histological appearance of the mast cells.
- b) Tenth day after irradiation. Disruption of the cell membranes with dispersion of the granules in two cells, and vacuolation in another mast cell.

Effect of irradiation on biological processes of the skin.

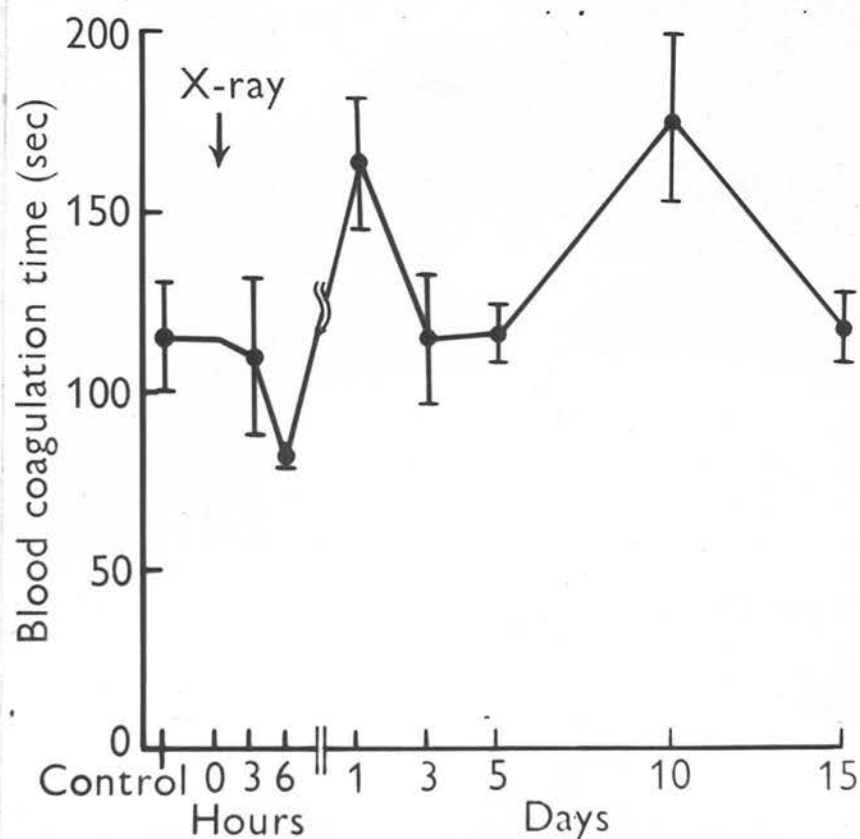


Fig. 17. Effect of 1025 r total body X-irradiation applied to the skin of the back on the blood coagulation time. The standard error of the observations at each time interval is shown.

TABLE 15

Effect of irradiation on histamine content of the skin.

Type of Irradiation	Dose	Histamine % of normal	Days after Irradiation
X	605	58	10
X	1000	39	10
X	2000	39	7
β	3000	39	10
Skin, mast cells	5 days	10 days	Regression of cell numbers, degeneration, decrease in numbers.
Stomach, "	5 days	10 days	
Stomach, "	24 hours	24 hours	Degeneration of all types of cells
Jejunum, "	24 hours	24 hours	
Blood, coagulation time	No effect	No effect	No effect
Blood, heparin coagulation time (Cohn, 1954)	6 days	10 days	25 x control values
Skin, Microscopic appearance	5 days	10 days	Erythema and desquamation, proceeding to ulceration.



TABLE 16

Effect of total body irradiation on the histamine content and histological appearance of the tissues and on the blood coagulation time.

Time examined	Time of effect following Irradiation		Maximum Intensity
	Onset	Maximum	
Skin, histamine	5 days	10 days	58% normal
Stomach, "	? 3 hours	5 days	58% normal
Jejunum, "	3 hours	5 days	8% normal
Skin, mast cells	5 days	10 days	Rupture of cell membranes, degranulation, decrease in numbers.
Stomach, "	5 days	10 days	
Stomach, mucous membrane ?		24 hours	Degeneration of all types of cells
Jejunum, mucous membrane ?		24 hours	
Blood, coagulation time	No effect	No effect	No effect
Blood, heparin coagulation time (Cohn, 1952)	6 days	16 days	25 x control values
Skin, Macroscopic appearance	6 days	10 days	Erythema and desquamation, proceeding to ulceration.

It has been shown by Riley & West (1953) that a large portion of the histamine contained in the skin is present in the mast cells. In the present experiments degeneration of the mast cells in the subcutaneous tissue spreads appeared after a latent period of several days, at the time when the skin histamine value began to fall, and was most extensive 10 days after irradiation when the skin histamine was at its minimum value. As the cells in the subcutaneous tissue began to resume their normal appearance, so the skin histamine value began to rise again. These results resemble those described by Riley & West (1955a) following the injection of 'subacute doses' of compound 48/80 and suggest that the fall in the skin histamine may be associated with the destruction of the mast cells in the skin. Similar degenerative changes have been reported after irradiation in the subcutaneous mast cells of the rat (Sylvén, 1940) and in the cheek pouch of the hamster (Smith & Lewis 1953). Increasing the dose of radiation in the rat, which has been shown by Bloom (1948) to cause increased destruction of mast cells, produced a slightly greater fall in the skin histamine.

Complete disappearance of the mast cells such as occurs after administration of compound 48/80 (Riley & West 1955) was not observed after either  $\beta$ - or X-irradiation and correspondingly the histamine content of the skin did not fall to the low values obtained after administration of compound 48/80. A comparison of the histamine liberating properties of irradiation and compound 48/80 indicates that the former, in the fairly large doses used in the present experiments, is less efficient than compound 48/80. The fact that their combination could not decrease the histamine content to a lower level than that obtained by compound 48/80 alone shows that irradiation, like compound 48/80, is unable to release the residual histamine in the skin which is resistant to release by any known liberators (Brocklehurst, Humphrey & Perry, 1955).

No significant change in the blood clotting time was observed at any stage. However, the finding by Cohn (1952) of a change in the heparin clotting time at the same time as a decrease in the skin histamine and degeneration of the mast cells following irradiation was observed in the present experiments, makes it probable that the changes in the mast cells determine both the changes in heparin clotting time and in the skin histamine.

Feldberg & Talesnik (1953) have shown that the alimentary canal is resistant to the action of the histamine liberators. That histamine can be held in the tissues independently of the mast cells is suggested by the fact that large doses of compound 48/80 and other liberators (Riley & West 1955b; Brocklehurst, Humphrey & Perry, 1955) sufficient to cause complete destruction of the mast cells do not deplete the skin completely of histamine. In the jejunum and stomach, the histamine values decreased several days before the gross changes in the mast cells occurred, which Riley & West (1955a) report are correlated with changes in histamine content. It therefore

appears that irradiation can deplete the alimentary canal of histamine independently of its action on mast cells, and that the histamine in the upper part of the alimentary canal may be held in the tissues of the wall other than in the mast cells.

It is claimed that one of the physiological functions of histamine in the gastric wall is the maintenance of the acid secretion by the parietal cells of the gastric mucosa (Code, 1956). A decrease in this acid secretion in human beings has been described by Palmer & Templeton (1939) following irradiation of the stomach. The mechanism by which irradiation causes this effect is unknown, but if irradiation causes a depletion of gastric histamine in man as it does in the rat, it is possible that the effect of irradiation on gastric secretion may be explicable in terms of its action on the histamine content of the stomach wall.

The dry weights of the stomach and intestine decrease and the water content increases in rats between the first and ninth days following irradiation (Bowers & Scott, 1951a; Conrad, 1952), but the percentage increase in water content is insufficient to account for the much larger percentage decrease in the histamine content of the tissues. A fall and then a rise takes place in the sodium and potassium contents of the stomach and intestine during the nine days following irradiation (Bowers & Scott, 1951a, 1951b), but these changes are not correlated in any way with the steady decrease in histamine content. The protein content of the alimentary canal also diminishes following irradiation with neutrons at the same time and over the same period as the intestinal histamine decreased in the present experiments (Ross & Ely, 1949). The changes in the histamine content following irradiation therefore appear to be related more closely to changes in the weight and protein content of the alimentary canal than to changes in its water or electrolyte content.

It has been shown in the present experiments that X-irradiation damaged the cells lining the stomach and jejunum. Cellular destruction of a similar nature has also been reported in these tissues by Pierce (1948) soon after exposure to radiation. These changes appeared at the same time as the tissue histamine began to decrease, but tissue repair was almost complete when the histamine content was still at its minimum.

The administration of chloramphenicol to the rat can reduce the histamine content of the wall of the small intestine to about 35% of normal through its action on the histamine-forming bacteria of the intestine (Wilson, 1954). Irradiation causes changes in the bacterial flora of the caecum in the rat (Bell, Coniglio & Hudson, 1955), and it is thus possible that the changes in the present experiments might be attributable to the effect of irradiation on the bacterial flora of the intestine. However, this could not account for the changes observed in the gastric histamine content, and it is unlikely that it would



affect any histamine-producing bacteria at the proximal end of the jejunum so grossly as to decrease the histamine in the wall of the jejunum by 90%. Exposure to X-irradiation is known to reduce the activity of cholinesterase in the rat intestine (Conard, 1952), and it is possible that it may influence the activity of the intestinal histaminase or histidine decarboxylase. In this way, by a primary effect on the enzymes responsible for the metabolism of histamine in the intestine, it may cause the observed changes in the intestinal histamine content.

No characteristic signs of histamine release were observed at any time following irradiation. The signs of histamine release appear after the injection of a large dose of compound 48/80 which produces its effects on the mast cells within 3 hours (Riley & West, 1955a). The gradual decrease in tissue histamine which was observed in the present experiments may account for the absence of clinical signs of acute histamine release.

The macroscopic changes observed in the skin following  $\beta$ -irradiation were similar to those described by Ungar & Damgaard (1954b). Bloom (1948) has described the microscopic changes in mice and rats which occurred at various periods after  $\beta$ -irradiation with 2500 and 5000 rep. and reports similar changes with both doses though they occurred slightly earlier with the larger dose.

The effect of histamine and compound 48/80 on the vascular permeability was examined in the presence of pontamine sky blue since it has been stated by Miles and Miles (1953) that this is the most suitable method to obtain reliable results. Ungar and Damgaard (1954b) have found that the whealing response of  $\beta$ -irradiated skin to compound 48/80 is inhibited during the period of six hours to nine days after irradiation although histamine elicited a normal response during this period. No dye was used for examining the reactions in their experiments. Twenty hours after irradiation in the present experiments, changes in the mast cells of the skin had begun to appear and the skin histamine content was just beginning to decrease, but it is clear from the similarity of the responses of normal and irradiated skin to the intradermal injection of histamine, that cutaneous vessels in the irradiated skin still reacted normally to histamine. At this time also, compound 48/80 produced a normal cutaneous vascular response and there was no interference with the mechanism by which compound 48/80 releases histamine. Miles & Miles (1953) have shown that compound 48/80 causes changes in vascular permeability by virtue of its histamine-liberating properties. Therefore, at this time, compound 48/80 was capable of liberating histamine in the normal fashion from the skin, and the vessels responded to this histamine in the normal way. Furthermore, it was found in the present experiments that in skin partially depleted by  $\beta$ -irradiation, compound 48/80 could still release sufficient histamine to produce a vascular response similar to that caused by histamine.



In animals which had previously been depleted of histamine by the administration of compound 48/80, the histamine content of the skin did not differ in irradiated and non-irradiated areas. In both areas, it was reduced to about 10% of the normal value. No differences were detected in the time of onset or the course of the macroscopic radiation lesions between normal and depleted animals, and recovery of skin histamine in irradiated areas was similar to that in non-irradiated areas. Ulceration caused by  $\beta$ -irradiation can therefore occur in the absence of 90% of the skin histamine, and histamine restoration in the irradiated areas proceeds at a normal rate. A similar observation was made with regard to the occurrence of passive cutaneous anaphylaxis and the Schwarzman reaction in depleted animals by Brocklehurst, Humphrey & Perry (1955).

Irradiation, as assessed by its effect on the histamine content of the tissues clearly causes changes in the metabolism and turnover of histamine. This effect precedes, and may occur independently of, the changes in the mast cells; moreover, the depletion of tissue histamine occurs more slowly than is the case with the sudden explosive effect of compound 48/80. Irradiation, unlike any of the other liberators, can deplete the alimentary canal of histamine. At the same time, its maximum capacity to deplete the skin is significantly less than that of compound 48/80. These observations suggest that different mechanisms may be responsible for the tissue depletion of histamine after irradiation and the administration of compound 48/80.

It has been pointed out already that irradiation may produce its effects by interfering with the enzymes which metabolise histamine, particularly in the alimentary canal which it has been shown contains high concentrations of histaminase and histidine decarboxylase in the rat (Watson, 1956). The gradual and incomplete fall of skin histamine suggests that irradiation caused histamine depletion here also by destroying the tissue enzymes responsible for producing or binding the histamine. The sudden loss of tissue stores of histamine associated with the explosive break-up of mast cells was retarded, and it appears possible that the depletion of tissue histamine was preceded by a disturbance of the integral processes associated with the metabolism of endogenous histamine. In the alimentary canal, the depletion proceeded to a large extent independently of the break-up of the mast cells. After irradiation, the loss of tissue histamine appears to depend on interference with tissue enzymes involved in the production and binding of histamine, and on destruction of mast cells. The rise in blood histamine which occurs after irradiation (Weber & Steggerda 1949) therefore probably originates from disintegration of mast cells and also from disturbance of the activity of the tissue enzymes responsible for the binding and catabolism of histamine. It has already been shown that these enzymes are particularly active on the alimentary canal and other abdominal organs (Chapter 3).

Tissue damage occurred rapidly in the alimentary canal; it reached its maximum and repair had commenced during the period when histamine depletion was still taking place. Commencement of depletion probably started at about the same time as the tissue damage, but the fact that depletion, associated with local histamine release, continued during the repair process suggests that the histamine did not play any important part in initiating or maintaining the damage.

Macroscopic and microscopic damage occurred in the skin when its histamine content began to diminish. However, it has been shown that irradiation does not interfere with the reactivity of the blood vessels to histamine, and that the severity of the skin damage was not diminished when the skin contained no histamine capable of being released. It can thus be concluded that radiation damage is brought about by a mechanism which is independent of intrinsic skin histamine.

Ulcers. Numerous studies have been made of the association of high acid secretion and ulcer formation. The disappearance of the gastric mucosa, which is the first step in the development of an ulcer, is associated with a reduction in the histamine content of the gastric mucosa. Following a review of the literature, it was concluded that whatever the initial mechanism of ulcer formation, the final mechanism must be related to the depletion of gastric histamine. Thus, in the absence of direct circumstantial evidence that histamine is responsible for the development and progress of gastric ulcers.

Hypersensitivity and gastric ulcers have been produced experimentally in the presence of low levels of histamine (Ray, Varco, Gude & Macpherson, 1951). In these experiments, hypersensitivity produced the appearance of ulcers and ulcer formation has accordingly been interpreted as a reaction of the gastric wall by the powerful self-digestion action of acid in the gastric lumen. It is not possible to determine the relationship between the secretion of acid and the formation of gastric ulcers experimentally by the technique of histamine depletion since an increase in the concentration of acid is likely to occur when the ulcers appear.

Investigation therefore, of ulcer formation without of producing ulcers would be valuable, to find out whether ulcers can only be produced in the absence of important gastric secretion, and whether or not histamine is the substance responsible for initiating the disease. There have been several clinical reports of the disappearance of gastric ulcers following the administration of antihistamines. This evidence in animals (Kirsner, 1951) and evidence in man (Kirsner & Ford, 1952) makes it clear that pathological changes can be reversed by physiological dysfunction which precedes the ulcer and following the administration of antihistamines. These reports, however, may be interpreted as evidence that made on the basis of the fact that antihistamines inhibit secretory functions and on the fact that ulcers are associated with

CHAPTER 5

THE ROLE OF ENDOGENOUS HISTAMINE IN GASTRIC ULCERATION

It has been stated that histamine is the final common local chemostimulator of the parietal cells of the gastric mucosa and considerable experimental evidence confirms the accuracy of this statement (Code 1956). There is also some evidence that histamine can cause an increased output of pepsin (Ashford, Heller & Smart, 1949; Watt & Wilson, 1958), and changes occur in the concentration of the other electrolytes in the gastric secretion (James, 1957). The occurrence of ulcers in the stomach has frequently been related to changes in the gastric secretion, and indeed it is commonly supposed that the presence of acid in the stomach is associated with the production of ulcers. Numerous clinical and experimental reports based on the association of high acid concentrations with ulcers, and the disappearance of the ulcers when the acidity of the gastric contents is reduced, support this idea (Varco *et al.* 1941; Pickering & James, 1949; Mann, 1951). Under these circumstances, whatever the initial mechanism of the increased gastric secretion, the final mechanism must be mediated through the endogenous gastric histamine. Thus, in the stomach, there is considerable circumstantial evidence that histamine is responsible for the development and progress of gastric ulcers.

Hyperacidity and gastric ulceration can easily be produced experimentally in the guinea-pig by the injection of histamine (Hay, Varco, Code & Wangenstein, 1942). In such experiments, hyperacidity precedes the appearance of the ulcers and ulcer formation has accordingly been attributed to digestion of the gastric wall by the powerful and unopposed action of acid in the gastric lumen. It is not possible to determine the relationship between the secretion of acid and the occurrence of gastric ulcers experimentally by the injection of histamine, since an increase in the concentration of acid is always present when the ulcers appear.

Investigation therefore, of other experimental methods of producing ulcers would be valuable, to find out whether ulcers can only be produced in the presence of increased gastric secretion, and whether acid actually is the constituent responsible for initiating the ulcers. There have been frequent clinical reports of the occurrence of gastro-intestinal symptoms following the therapeutic use of phenylbutazone. Experimental evidence in animals (Bonfils, Hardouin & Delbarre, 1954; Kirsner & Ford, 1955a) and evidence in humans (Mauer, 1955) now makes it clear that pathological changes and a variable degree of physiological dysfunction take place in the stomach during and following the administration of this drug. In spite of these reports, however, very few experimental observations have been made on the changes which phenylbutazone produces on gastric secretory function and on the time during which such changes

persist. Bonfils, Hardouin, Richir, Delbarre & Lambling (1955) have reported that it causes achlorhydria in rats but that it has no effect on human gastric secretion. Kirsner & Ford (1955a, 1955b) found that it caused an increased secretion of acid in some of their experiments with dogs and an increased basal secretion in some of their investigations in man. In the present experiments, an investigation of the properties of phenylbutazone in the guinea-pig has shown that the onset and progress of pathological processes in the stomach can be correlated with changes occurring in the gastric secretion, and some further light has been thrown on the relationship between the appearance of ulcers and the action of endogenous histamine in the gastric wall.

The advantages of using guinea-pigs in large numbers for the investigation of ulcerogenic agents, and of factors influencing the formation of ulcers, are well recognised (Hay, Varco, Code & Wangenstein, 1942; Harrison, Packman & Goldberg, 1956). Nevertheless, the physiology of the gastric secretion in the guinea-pig has until recently received little detailed study. The technique used in the present experiments enabled changes in all the constituents of the gastric contents to be measured in the unanaesthetized animal. The measurements were made before any experimental procedure was started and at frequent intervals throughout the subsequent experiments. The concentrations of electrolytes and the peptic activity of the gastric secretion were examined. The measurements were made after a period of fasting and also after the secretory power of the cells had been stimulated maximally by histamine or methacholine. The effect of phenylbutazone on these secretory responses was then investigated, and the period over which the changes persisted in the different elements of the secretion was observed.

The morphological changes and the extent of the gastric damage induced by histamine and phenylbutazone were examined by macroscopic examination and standard histological procedures. The chemical methods used for the investigation of the gastric secretion and the methods employed for examination of the pathological changes in the stomach are described in Chapter 2. In all the experiments, a group of at least 6 guinea-pigs was used to make each observation, and generally, the observations were derived from the mean of the results from much larger groups.

#### THE FASTING SECRETION AND APPEARANCE OF THE NORMAL STOMACH.

In all the guinea-pigs, gastric secretion was removed by stomach tube at various periods after administration of the drugs, and some of the animals were finally killed in order to determine the extent of the gastric damage. The secretion was removed at the time of death and analysed at the same time as



the other samples. A small quantity of fasting secretion was always found in the stomach after 16 hours fasting. The peptic activity and concentrations of the acid and other electrolytes in this secretion were used as the standards with which those in the drug-induced secretions were compared.

The fasting secretion was collected from a group of fourteen animals. The volume of secretion was 4.2 ml with a standard error of 0.5 ml. Very few particles of white mucus were seen and the fluid itself did not have the sticky feeling which was often associated with secretion obtained after methacholine.

Since solid food or faeces in the stomach is more likely to block the tube in fasting animals than in animals in which the secretion has been stimulated by drugs, six of the guinea-pigs were killed immediately after removal of the secretion in order to determine how much juice had been left in the stomach. It was found that the volume of residual juice ranged from 0.2 to 1.0 ml. As a further check on the accuracy of the intubation method, the stomach contents were removed post-mortem from a second group of fourteen fasting animals. The mean volume of juice so obtained was 4.8 (S.E. 0.5 ml).

The results showed that the difference between the mean volumes collected by stomach-tube and at post-mortem is not significant. The constitution and volume of the gastric secretion obtained post-mortem from the second group of animals is shown in Table 17 and compared with the constitution of the secretions elicited by histamine and methacholine one hour after the drugs had been injected. At post-mortem, the oesophagus and stomach of these animals were closely examined for signs of damage produced by passage of the stomach-tube. There was slight hyperaemia round the cardiac orifice in three of the guinea-pigs, but no other damage was observed in the stomachs. In none of the stomachs was any acute or chronic erosions or ulcers seen.

#### THE EFFECT OF HISTAMINE ON GASTRIC FUNCTION AND PATHOLOGY.

The histamine secretion: Following the injection of histamine, the stomach tube was easy to pass and a very large volume of fluid secretion siphoned out. No mucus was seen, and unless the guinea-pig had indulged in excess coprophagy, the secretion was almost clear. Less than 10% of the gastric contents consisted of solid matter. To ascertain that complete emptying of the stomach was being secured, a few animals were killed immediately after intubation; a range of only 0.1 - 0.7 ml of residual juice was found. The volume and constitution of the histamine secretion one hour after intramuscular injection of histamine acid phosphate in beeswax are shown in Table 17. A large volume of juice with a high concentration of acid and

TABLE 17

The mean volumes and constitution of the gastric secretion obtained in response to various stimuli. The numbers of animals and standard errors are indicated. Acid and electrolytes in m-equiv/l. Histamine, I.M.I., Methacholine, 6 injections I.P.I.

	Fasting	Stimulus Histamine 10 mg/kg.	Methacholine 0.02 mg/kg.
No. of Animals	14	25	6
Vol. (ml)	4.86 $\pm$ 0.52	18.40 $\pm$ 0.98	5.86 $\pm$ 0.98
Solid matter %	17	7	18
Pepsin units/litre	600 $\pm$ 100	1110 $\pm$ 123	2080 $\pm$ 470
Free acid	69 $\pm$ 5	103 $\pm$ 4	83 $\pm$ 6
Na	29 $\pm$ 4	19 $\pm$ 1	23 $\pm$ 3
Cl.	93 $\pm$ 5	140 $\pm$ 5	113 $\pm$ 5

Differences in the volume, free acid and Na between the fasting and methacholine secretions, and the difference in the Na between the methacholine and histamine secretions, are not significant. All other differences are significant at a value of  $P < 0.05$ .

Macroscopically, the ulcerated areas were classified as non-haemorrhagic or haemorrhagic. The large non-haemorrhagic ulcers, in the 24 hour group, the cells had been degenerated as far as the muscularis mucosae, and this was showing early signs of degeneration (Fig. 185). The walls of the ulcers were steep and red blood cells were present among the necrotic debris of the ulcers (Fig. 186). These had already leaked out of the ulcers and were not yet in contact with the surrounding tissue during the development of the ulcer. The cells in the ulcers in the 48 hour group were not yet degenerated and the epithelium did not appear to be abnormally thickened or congested.

Individual ulcers were present in three of the 14 animals in the 24 hour group and the morphology and progress of the ulcers was similar to that in the 48 hour group. The ulcers had steep sides and the epithelium had degenerated, and frequently the ulcers had progressed to the point where the muscularis mucosae was exposed.

low  $\text{Na}^+$  concentration was obtained. The concentration of pepsin was slightly higher than that in the fasting juice but because a large volume of juice was secreted, the pepsin content in the stomach 1 hour after administration of the drug was as high as that following the administration of methacholine. Twelve and 24 hours after the guinea-pigs had received the histamine, the gastric juice obtained from some of them was stained with faeces because they had been indulging in coprophagy during the period of fasting. The acid concentration was still greatly raised in comparison with the fasting juice when they were killed after 24 hours, although the concentration of total acid was increased at the expense of free acid in those which had been eating faeces.

Histamine-induced ulceration: Gastric and duodenal ulcers had begun to appear 12 hours after the guinea-pigs had received the histamine in beeswax. A dose of 20 mg/kg was given by intramuscular injection under cover of mepyramine. This dose of histamine is normally lethal in all the guinea-pigs, but no deaths occurred in these experiments on account of the protection by the anti-histamine.

Three kinds of gastric damage appeared in the stomachs; dull white areas of superficial necrosis, non-haemorrhagic and haemorrhagic ulcers. Dull white areas were present on the surface of the mucosa of one of the animals killed after 12 hours and were also scattered amongst the ulcerated areas in those killed at 24 hours. Histologically, these areas showed various stages of degeneration and necrosis of the superficial cells of the mucosa. In some places, it could be seen histologically that these cells had separated from the mucosa and early ulcers had formed (Fig. 18a).

Macroscopically, the ulcerated areas were classified as non-haemorrhagic or haemorrhagic. The large non-haemorrhagic ulcers were sometimes as much as 1 - 2 mm in diameter and were easily identified as small craters with irregular bases, sometimes with a brown stain in them. The haemorrhagic ulcers were of a similar size but the craters were filled with blood which could be washed away with some difficulty. Histologically, the ulcers showed degeneration and necrosis of the mucosa, and frequently, in the 24 hour group, the cells had been desquamated as far as the muscularis mucosae, and this was showing early signs of degeneration (Fig. 18b). The walls of the ulcers were steep and red blood cells were present among the necrotic cells of the mucosa (Fig. 18c). These had clearly leaked out of the mucosal capillaries as they had become involved in the damage during the development of the ulcer. The cells in the mucosa in the undamaged areas showed no abnormalities and the capillaries did not appear to be abnormally dilated or congested.

Duodenal ulcers were present in three of the ten animals and the morphology and progress of the ulcers was similar to that in the stomach. The ulcers had steep sides, some bleeding had occurred, and frequently damage had progressed as

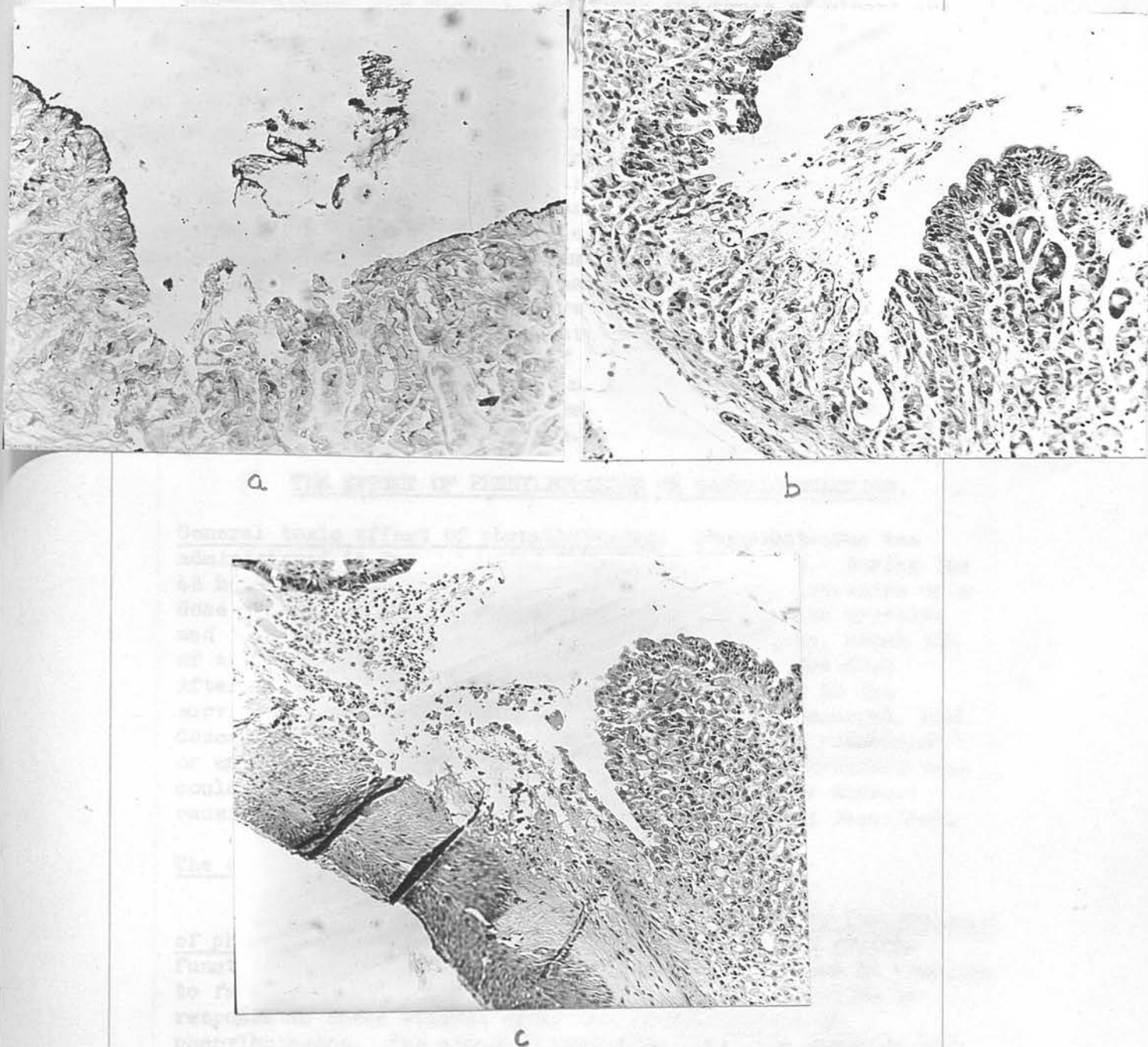


Fig. 18. Sections of stomach-wall of the guinea-pig 24 hours after receiving histamine acid phosphate in beeswax, 20 mg/kg I.M.I.

- a) Early stage of histamine damage showing superficial degeneration and necrosis. Mucicarmin and P.A.S.
- b) Later stage of histamine ulcer showing exposed blood vessels and steep walls of ulcer. H. & E.
- c) Base of well-developed ulcer showing degeneration of muscularis mucosae. H. & E.



far as the muscularis mucosae which showed signs of degeneration. The numbers, positions and types of ulcers are summarised in Table 18 and the values for the concentration of acid in the gastric contents are shown at the time of death.

All the animals except one had ulcers in the stomach or duodenum. Ulcers occurred in both the stomach and duodenum in only one guinea-pig. The concentration of acid in the stomach at the time of death was high in all the guinea-pigs, but the individual values did not appear to be related to the number or position of the ulcers (Table 18). There was no difference in the number of ulcers per animal occurring in the 12 or 24 hour groups. It is clear that the initial weakness which led to the development of ulceration in the gastric or duodenal mucosa appeared within the first 12 hours after injection of the drug, and then, in spite of the continued secretion of acid into the lumen of the stomach, no significant increase in the number of ulcers occurred between 12 and 24 hours after administration of the histamine.

#### THE EFFECT OF PHENYLBUTAZONE ON GASTRIC FUNCTION.

General toxic effect of phenylbutazone: Phenylbutazone was administered in doses ranging from 50 to 225 mg/kg. During the 48 hours following a single intraperitoneal administration of a dose of 200 or 225 mg/kg, the guinea-pigs lost their appetite and their weight diminished. With the larger doses, about 10% of the animals died within 48 hours of receiving the drug. After 48 hours, the appetite and general condition in the surviving animals improved and no further deaths occurred. With doses below 200 mg/kg toxic effects were much less pronounced or entirely absent. No diarrhoea was observed. Phenylbutazone could be given again in a week after the first dose without causing any effects more serious than those already described.

#### The effect of Phenylbutazone on gastric secretion.

The gastric response to standard stimuli in the presence of phenylbutazone: The effect of phenylbutazone on gastric function has been assessed by comparing the secretion in response to fasting, histamine or methacholine with the secretion in response to these stimuli after the administration of phenylbutazone. The standard responses have been compared with the secretory response of the stomach 2 hours after the intraperitoneal injection of phenylbutazone, at which time it has been shown that its maximum pharmacological effect occurs (Domenjoz, 1952, 1953), and examined during the remainder of the period while it remained abnormal.

The intraperitoneal injection of phenylbutazone into the fasting guinea-pig caused a considerable reduction in the acidity of the juice after 2 hours. A dose of 200 mg/kg reduced the concentration of free acid from the fasting level of

TABLE 18

Concentrations of acid and incidence of gastric and duodenal damage in individual guinea-pigs following 20 mg of histamine acid phosphate in beeswax, I.M.I. Acid in m.equiv/litre. H., Haemorrhagic ulcers; N.H., Non-haemorrhagic ulcers.

Free Acid	Total Acid	Gastric Ulcers H	Gastric Ulcers N.H.	Duodenal Ulcers	Remarks
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Killed 12 hours after injection:

108	120	2	1	0	Necrotic areas
66	110	0	0	3	
12	84	2	9	0	
68	120	1	0	0	

Killed 24 hours after injection:

80	116	6	0	0	
110	126	0	3	3	Necrotic areas
110	124	0	0	2	
90	140	0	0	0	
118	134	5	6	0	Necrotic areas
72	102	3	1	0	

Killed 1 hour after injection:

103	122	0	0	0	Mean of 10 Animals.
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The effect of phenylbutazone on secretion of electrolytes. Phenylbutazone in a dose of 200 mg/kg caused a considerable increase in the concentration of sodium in the gastric secretion. The sodium concentration was two and a half times the normal fasting value after 2 hours, and it rose to a maximum 1 hour after administration of the phenylbutazone at the time when the concentration of free acid was at its minimum value. The sodium concentration in the gastric juice then diminished and approached the normal fasting level after 18 hours at the same time as the acidity of the juice returned to the fasting value (Fig. 20). Phenylbutazone in fasting guinea-pigs did not cause any change in the concentration of chloride in spite of the fact that it caused a reduction in the mucous concentration and an increase in the concentration of sodium (Table 20).

69 (S.E. <sup>+</sup> - 5) to 19 (S.E. <sup>+</sup> - 5) m.equiv/litre; about two-thirds of the animals in the different experiments had no free acid in their gastric secretion. Smaller doses produced less effect and no significant effect was caused by a dose of 50 mg/kg (Fig.19). The lowest concentration of free acid was found 3 hours after administration of the phenylbutazone (200 mg/kg) when the juice contained only 5 m.equiv/litre; five out of six animals having no free acid in the gastric contents. The phenylbutazone continued to have a pronounced effect until 14 hours after its injection. The concentration of free acid then rapidly returned towards the normal fasting level which was reached after a further 4½ hours (Fig.20).

The ability of phenylbutazone to reduce the concentration of free acid in the gastric secretion appeared in histamine-induced as well as in fasting secretion. Its intraperitoneal injection one hour before the injection of histamine completely inhibited or very greatly reduced the subsequent acid secretion. The normal response of the stomach to the injection of histamine was completely abolished by the largest dose of phenylbutazone in the majority of the animals, and smaller doses had less pronounced effects (Table 19). The reduction in the concentration of free acid in the secretion in response to histamine persisted for 6 hours. At 9 hours, the concentration of free acid was beginning to increase and 14 hours after the injection of phenylbutazone, it had almost returned to normal. After 17 hours, the gastric secretion contained the usual high concentrations of acid (Fig. 21).

Reappearance of acid in the gastric juice in fasting guinea-pigs and in those which had received histamine, occurred 12 - 14 hours after the injection of the phenylbutazone, and in both groups, no impairment in the acid-secreting power of the stomach could be detected after 18 hours. This rapid and complete reversal of the inhibitory effect of phenylbutazone indicates that any damaging effects which the drug may have on the parietal cells are only temporary and do not permanently interfere with their function.

The effect of phenylbutazone on secretion of electrolytes: Phenylbutazone in a dose of 200 mg/kg caused a considerable increase in the concentration of sodium in the gastric secretion. The sodium concentration was two and a half times the normal fasting value after 2 hours, and it rose to a maximum 3 hours after administration of the phenylbutazone at the time when the concentration of free acid was at its minimum value. The sodium concentration in the gastric juice then diminished and approached the normal fasting level after 18 hours, at the same time as the acidity of the juice returned to the fasting value (Fig.20). Phenylbutazone in fasting guinea-pigs did not cause any change in the concentration of chlorides in spite of the fact that it caused a reduction in the hydrogen concentration and an increase in the concentration of sodium (Table 20).

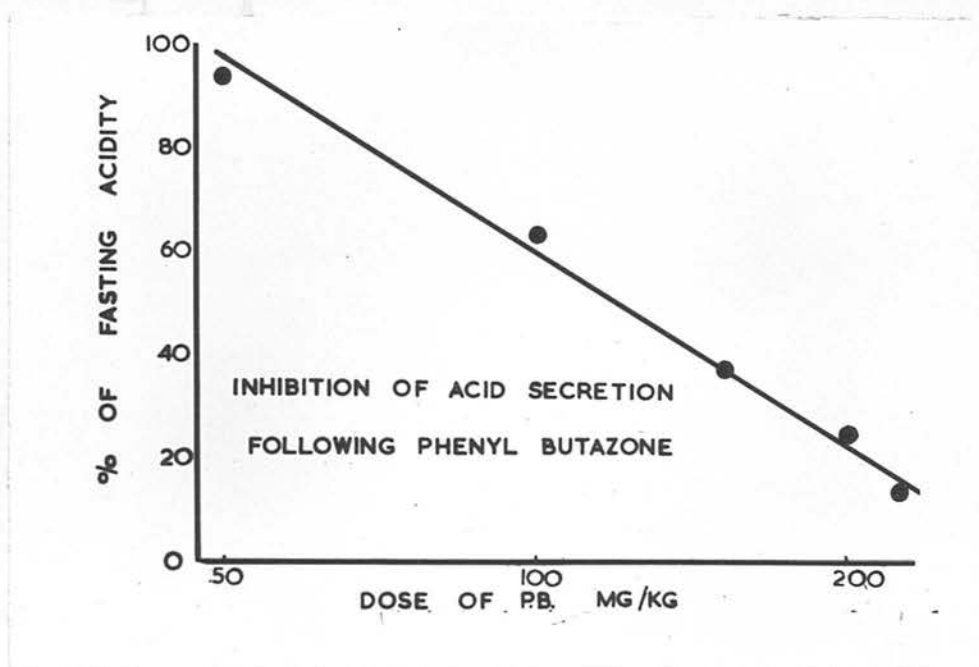


Fig. 19. The inhibitory effect of phenylbutazone on the secretion of free acid by the fasting guinea-pig two hours after injection of the drug.



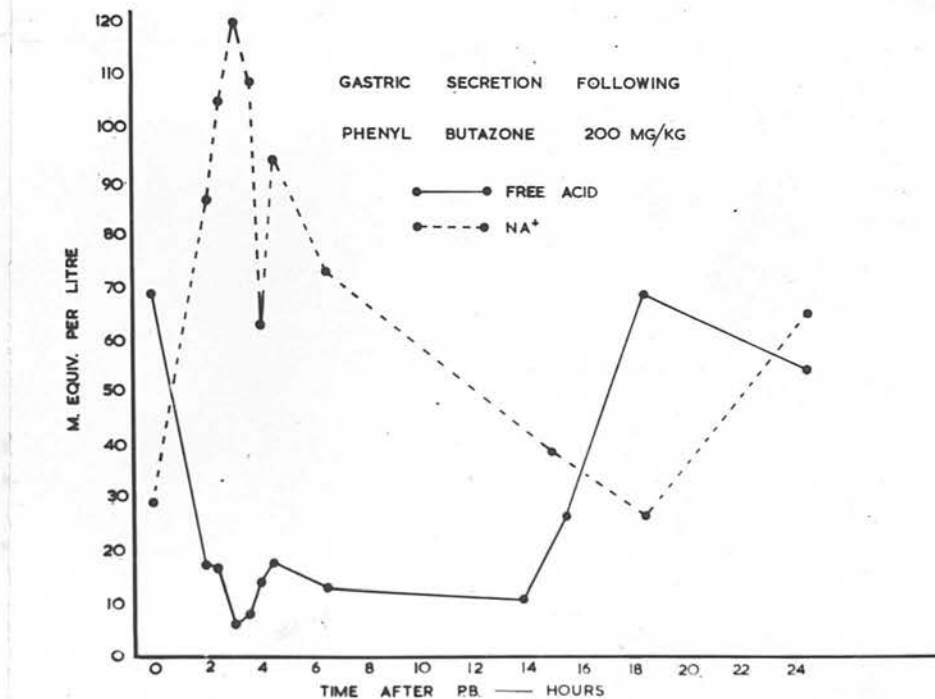


Fig. 20. The effect of phenylbutazone, 200 mg/kg I.P.I. on the concentrations of free acid and sodium in the gastric contents of fasting guinea-pigs.

TABLE 19

Effect of phenylbutazone on histamine-induced gastric secretion two hours after the injection of phenylbutazone.

Dose of Phenylbutazone	No. of Guinea-pigs	Volume ml	Free acid m.equiv/litre
50	2	5.6	65
100	3	7.9	53
150	3	5.0	8
200	13	5.2	8
225	6	7.2	6
Histamine alone	25	18.4	103

Fig. 21. The effect of phenylbutazone, 250 mg/kg i.p., on the values and observation of free acid in histamine-induced gastric secretion.

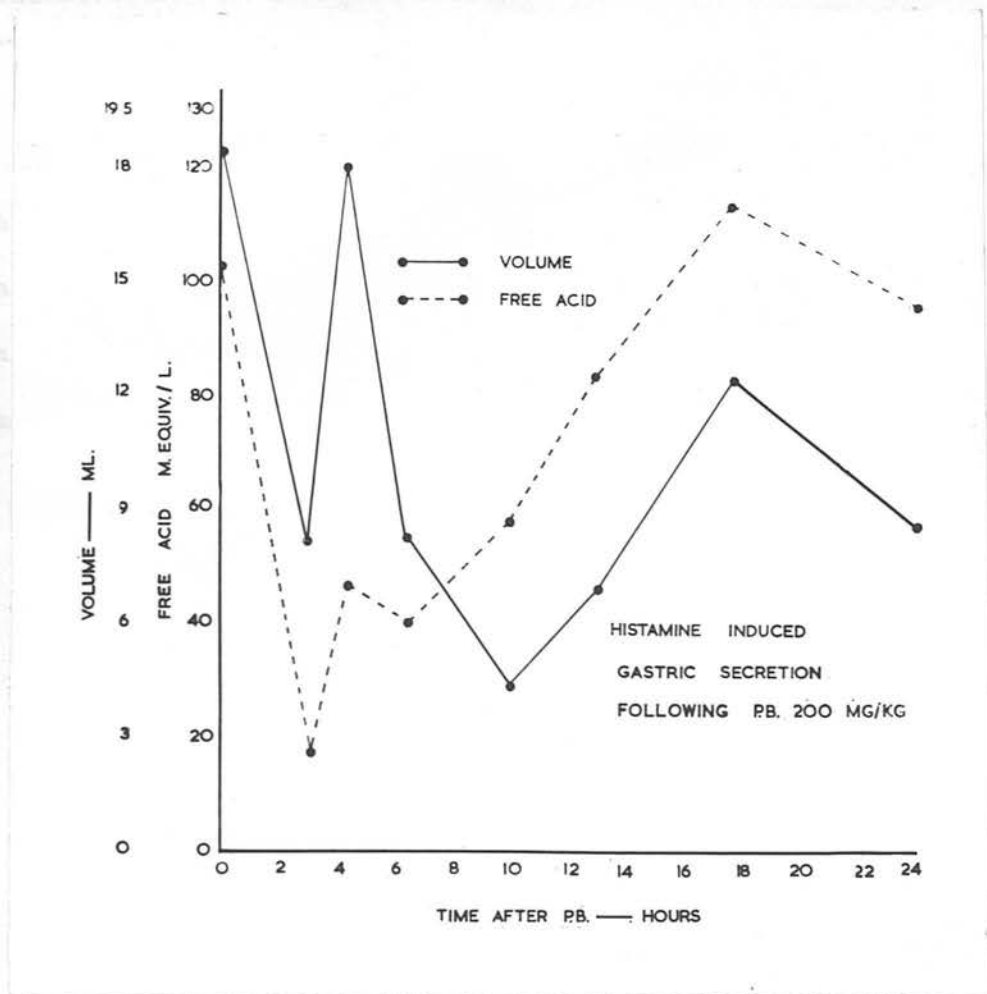


Fig. 21. The effect of phenylbutazone, 200 mg/kg I.P.I. , on the volume and concentration of free acid in histamine-induced gastric secretion.

TABLE 20

The electrolyte concentrations in the gastric secretion 2 - 3 hours after administration of histamine and phenylbutazone. Concentrations in m. equiv/litre.

Stimulus	H <sup>+</sup>	Na <sup>+</sup>	Cl <sup>-</sup>
Fasting	69 ± 5	29 ± 4	93 ± 5
Fasting + Histamine	103 ± 4	19 ± 1	140 ± 5
Fasting + Phenylbutazone	19 ± 5	84 ± 13	86 ± 5
Fasting + Phenylbutazone + Histamine	8 ± 3	78 ± 9	95 ± 5

The total quantity of free acid produced by the gastric cells 2-3 hours after the injection of phenylbutazone, in spite of the high volume of the gastric secretion, was only 4% of that present in the secretion from the normal fasting group, and the total quantity of sodium in the gastric juice was only one-sixth as much as is normally present. The total gastric activity of the gastric juice was greatly increased after administration of the phenylbutazone because an increase in volume of juice was associated with raised gastric activity.

#### Changes in Gastric Mucosa Following Administration of Phenylbutazone

**Morphology of gastric lesions:** Gastric lesions occurred with equal frequency on the anterior and posterior walls of the stomach. The lesions were more common in the lower two-thirds of the stomach, although the pyloric vestibule was seldom affected. Involvement of the duodenum was relatively uncommon.

Three types of gastric lesion were seen: superficial ulceration; non-haemorrhagic ulceration; and haemorrhagic ulceration.

While focal areas of superficial ulceration were common in the 1 and 4 hour groups and in the 12 and 24 hour groups, areas of gastric mucosa of various sizes and depths which appeared as pale, irregular patches on the surface of the mucosa were found in all groups. These areas were found in the lower two-thirds of the stomach and in the duodenum. In some cases the lesions were small and shallow, but in others they were deep and extensive.

Non-haemorrhagic ulcers occurred in the majority of the animals in the 12 and 24 hour groups. These ulcers were of the superficial type, although in some cases they were deep and extensive.



The effect of phenylbutazone on the peptic activity and on the volume of the gastric secretion: The peptic activity of the gastric contents was considerably raised above the fasting value 2 - 4 hours after administration of 200 mg/kg of phenylbutazone, but after the fifth hour, the activity decreased rapidly and from 14 - 24 hours after the injection, no peptic activity was found in the secretion of most of the animals (Fig.22). Seventy-two hours after administration of the phenylbutazone, the secretion obtained in response to 0.02 mg/kg of methacholine contained 62% of its normal peptic activity. The normal gastric response with high peptic activity following the administration of methacholine, as shown in Table 17 for normal animals, was found in six animals a week after the administration of phenylbutazone. Although complete functional recovery of the peptic cells ultimately occurs, it does not occur so quickly as in the case of the parietal cells.

Phenylbutazone caused an initial increase in the gastric volume, but this subsequently diminished and 6 hours after the injection, the volume became less than the normal fasting value.

The total quantity of free acid produced by the gastric cells 2½ hours after the injection of phenylbutazone, in spite of the high volume of the gastric secretion, was only 41% of that present in the secretion from the normal fasting guinea-pig, and the total quantity of sodium in the gastric juice was more than six times as much as is normally present. The total peptic activity of the gastric juice was greatly increased after administration of the phenylbutazone because an increased volume of juice was secreted with raised peptic activity.

#### CHARACTERS OF GASTRIC DAMAGE FOLLOWING PHENYLBUTAZONE ALONE.

Morphology of gastric damage: Gastric lesions occurred with equal frequency on the anterior and posterior walls of the stomach. The lesions were more common in the lower two-thirds of the stomach, although the pyloric vestibule was seldom affected. Involvement of the duodenum was relatively uncommon.

Three types of gastric lesion were seen macroscopically: superficial mucosal necrosis; non-haemorrhagic and haemorrhagic ulceration.

White focal areas of superficial mucosal necrosis were commonest in the 2 and 4 hour groups and in the 18 and 24 hour groups. Areas of the gastric mucosa of various sizes had a dull white appearance, but no interruption of the surface of the mucosa was observed and no blood was seen on the surface of the mucosa in association with these areas. In assuming the degree of gastric damage, these areas were not classified as ulcers.

Non-haemorrhagic ulcers occurred as frequently as the haemorrhagic type, although on cursory examination, they were

not so readily visible. Mucous membranes were exposed by gently peeling off dark red blood which could be pushed or washed off the surface of the mucosa with some difficulty. The non-mucous gastric ulcers in the 1-2 hour period were circular and air-dry in size. Although some small circular ulcers were found in the animals killed in the 12 hour and later groups, the ulcers in these animals tended to be larger and more irregular in outline, and included some lesions which sometimes showed branching. In these later groups, the hemorrhagic patches were also more extensive. Examination of the stomachs was found to be quite easy in the 24 hour group.

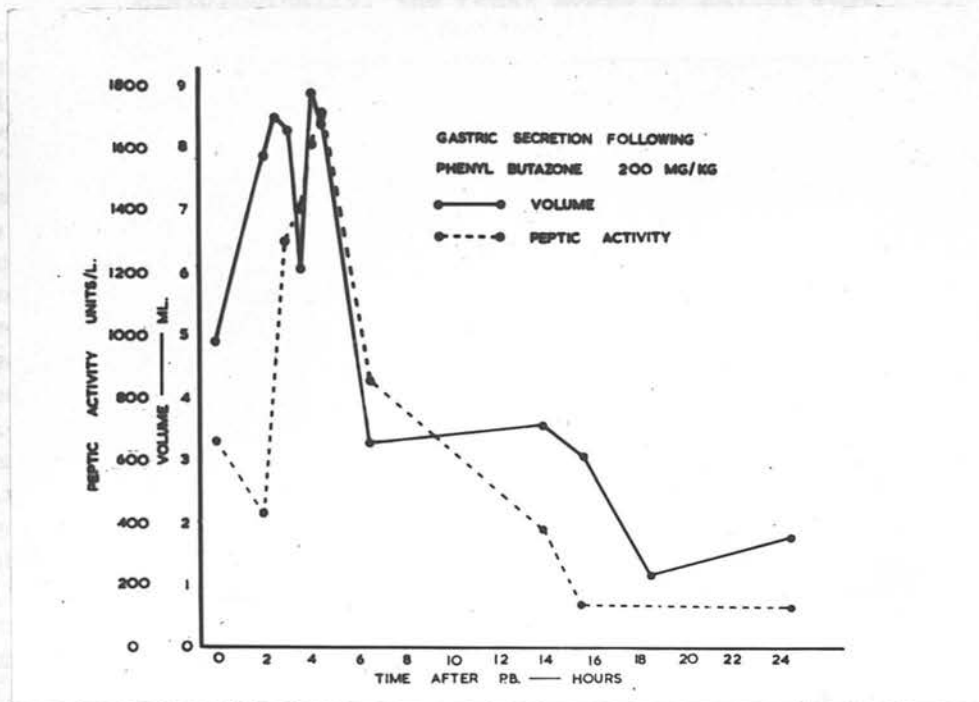


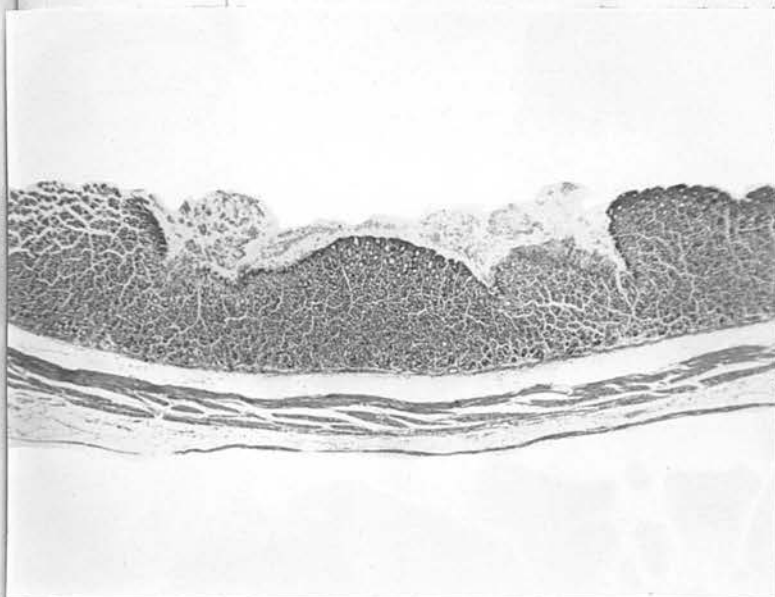
Fig. 22. The effect of phenylbutazone, 200 mg/kg I.P.I., on the volume and peptic activity of the gastric contents of fasting guinea-pigs.

not so readily visible. Haemorrhagic ulcers were surmounted by small patches of dark red blood which could be rubbed or washed off the surface of the mucosa with some difficulty. The non-haemorrhagic ulcers in the 2 - 4 hour period were circular and pin-head in size. Although some small circular ulcers were found in the animals killed in the 14 hour and later groups, the ulcers in these animals tended to be larger and more irregular in outline, and included linear lesions which sometimes showed branching. In these later groups, the haemorrhagic patches were also more extensive. Perforation of the duodenum was found in one guinea-pig in the 24 hour group.

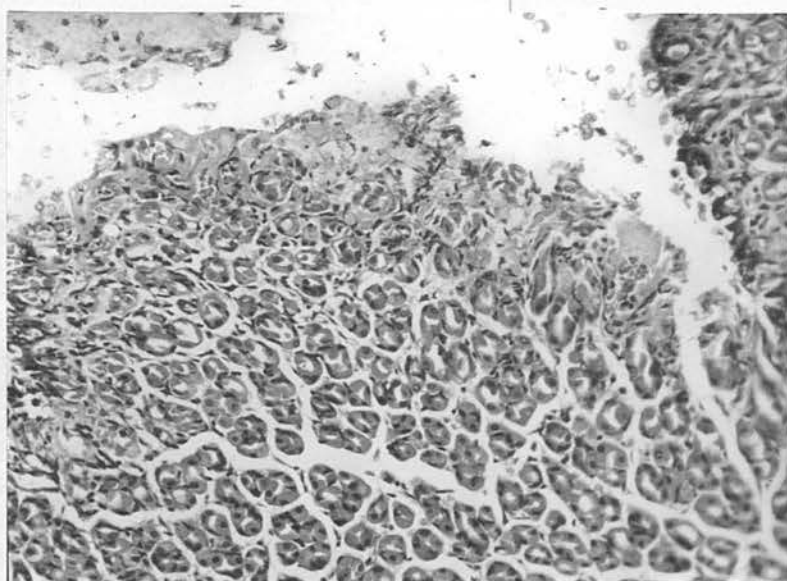
Histologically, the focal areas of pallor were identified as superficial lesions involving only the surface mucosal cells. There was loss of staining affinity and depletion of mucin. Some desquamation of the superficial cells was usually present, but no actual erosions were observed (Fig. 23a). Ulcerative lesions were mainly confined to the mucosa, although in some of the older lesions, the damage extended almost as far as the muscularis mucosae. Degeneration and necrosis of the cells on the floor and sides of the ulcer were observed and, not infrequently, the mucosal capillaries were exposed. Considerable congestion of the surface vessels was present in the damaged areas and leakage of the red blood cells onto the surface of the mucosa had occurred round the lesions in the 24 hour group (Fig. 23b). Interstitial haemorrhage did not occur in relation to the ulcers or elsewhere in the gastric mucosa. Slight polymorphonuclear infiltration was first seen in the 6 hour group and it was more extensive at the sites of the older lesions in the 18 hour and 24 hour groups, (Fig. 23b). No abnormalities were seen in the gastric mucosa except in the areas of necrosis and ulceration. No degenerative changes were present in the pepsin secreting or parietal cells (Fig. 23c).

The stomachs of the animals killed during the 4 - 14 hour period often appeared to be somewhat atonic when they were removed and the walls had a limp thin feeling. However, on histological examination, their muscle coats were perfectly normal.

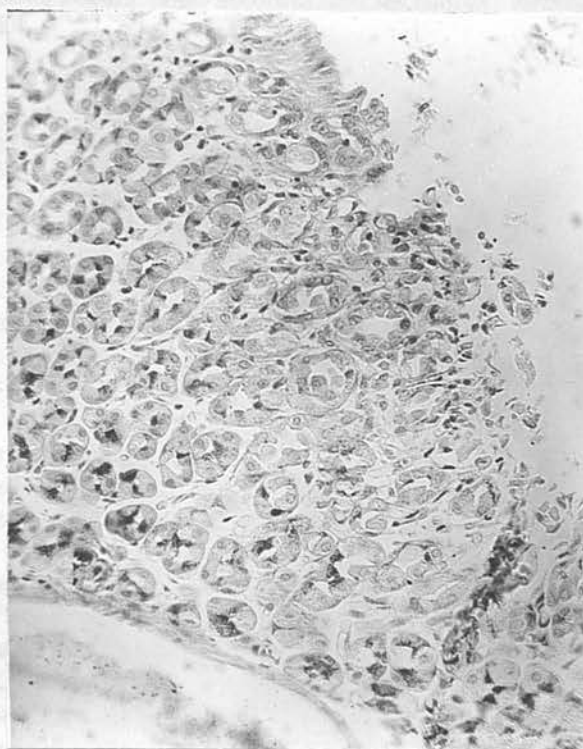
Incidence of damage and progress of ulceration: It is difficult to obtain an accurate assessment of the degree of gastric damage by simple macroscopic examination. However, by examining the gastric mucosa for signs of focal necrosis and by counting the total number of ulcers in each stomach, it was found possible to make a quantitative assessment of the progress of gastric damage from the time when the first signs appeared, 2 hours after the injection of phenylbutazone, until the end of the longest experiment after 24 hours. Where the configuration of the lesions suggested the confluence of several small ulcers, allowance was made for this when counting the total number of ulcers in the stomach.



a



b



c

Fig. 23. Sections of the stomach-wall of the guinea-pig after receiving phenylbutazone 200 mg/kg I.P.I.

- a) Early stage of gastric damage showing focal necrosis and desquamation of the superficial cells of the mucosa. H & E.
- b) Later stage of ulcer in guinea-pig killed 24 hours after injection of phenylbutazone. Penetration of ulcer into mucosa, and leakage of red blood cells from damaged capillaries. H & E.
- c) Gastric mucosa showing the pepsin granules and normal eosin staining of the parietal cells. Mann's methyl-blue and eosin.



Lesions were found in all the guinea-pigs which received phenylbutazone. In most of the animals, there were numerous ulcers, but in one killed after 14 hours, there were no lesions in the stomach and only one in the duodenum, and there was only one lesion in the stomach of one of the 24 hour group. Lesions were found in the duodenum in only 2 out of the 36 stomachs examined after the injection of phenylbutazone.

Numerous ulcers were found in the stomachs of the guinea-pigs killed 2 hours after receiving the phenylbutazone. The number of ulcers increased slightly until 6 hours after injections, but no significant change in the number occurred during the 6 - 14 hour period. Another significant increase occurred during the 18 - 24 hour period, a large number of large old ulcers and some new freshly formed small ulcers being present in the mucosa 24 hours after injection of the drug. The mean number of ulcers per animal in the 2 hour group has been compared with the number in the later groups in Table 21. A significant increase in the haemorrhage and extent of gastric damage only occurred early, and 18 - 24 hours after administration of the drug.

#### RELATION BETWEEN CHANGES IN GASTRIC SECRETION AND PROGRESS OF GASTRIC DAMAGE.

The relationship between the gastric secretion and the progress of gastric damage was examined in six groups of guinea-pigs, killed at intervals after the injection of 200 mg/kg of phenylbutazone. The gastric contents were removed from all these animals at post-mortem, so that no artifacts were produced in the gastric mucosa. In the following figures and tables, the values for the concentration of acid were obtained from these animals. The values for the peptic activity of the gastric contents were obtained from the animals described in the first part of this chapter.

Gastric damage appeared and the ulcers increased in number during the first 2 hours after injection of the phenylbutazone, when the concentration of acid in the gastric contents was diminishing (Table 21). At this time, the peptic activity in the stomach was also falling, owing to the rise in pH of the gastric contents. There was no significant change in the number of ulcers during the 2 - 14 hour period when the gastric acidity was almost zero, but, when the concentration of acid rose during the 18 - 24 hour period, the number of ulcers again increased significantly. During this period however, the peptic activity remained at an extremely low level (Fig. 24).

#### CHARACTER OF THE GASTRIC DAMAGE FOLLOWING PHENYLBUTAZONE AND MAGNESIUM OXIDE.

In view of the fact that gastric damage appeared and extended only during the periods when acid was present in the gastric contents, magnesium oxide was administered to the guinea-pigs prior to, and following, the injection of phenyl-

TABLE 21

The concentration of free acid and progress of gastric damage in groups of guinea-pigs after receiving phenylbutazone 200 mg/kg. Individual groups at each time period contained six animals. Free acid in m. equiv/litre. Means and standard errors of the collective groups are shown.

Time hours	Free Acid	No. animals without acid	Mean ulcers per animal		Significance
			Individual Groups	Collective Groups	
0	69	0	0	0	Sig.
2	8	4	29		
4	20	2	25	32	± 5.7
6	22	2	36		
14	11	2	37		
					Sig. t = 3.3
18	69	0	39	45	± 10.2
24	47	1	53		

Fig. 24. Peptic activity, concentration of free acid and incidence of gastric ulceration in guinea-pigs after receiving phenylbutazone 200 mg/kg i.p.

histamine in order to determine the effect of this substance on the gastric mucosa. Since the gastric damage appeared and increased markedly during the first six hours after administration of the phenylbutazone, when the acid concentration was falling from its normal fasting level almost to zero, the effect was given at hourly intervals during this period and the pH was measured at the end of each 2 hours. The acidity of the gastric contents was measured at the time of death with Topfer's reagent, and the specimens were classified as acid if the pH was less than 4.1 by this test.

In the group which received 20 mg/kg of H<sub>2</sub>, the incidence of ulceration was reduced in comparison to those which received

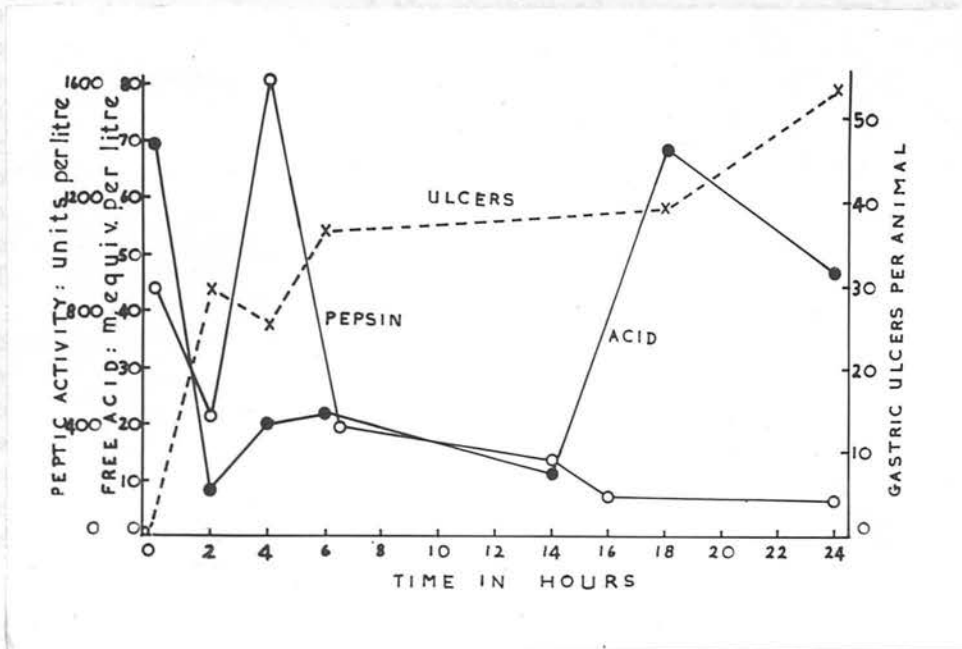


Fig. 24. Peptic activity, concentration of free acid and incidence of gastric ulceration in guinea-pigs after receiving phenylbutazone 200 mg/kg I.P.I.

ulcers can be produced in guinea-pigs by the ingestion of a histamine-releasing agent - but none developed and in some cases the effect is accompanied by the presence of a high volume of gastric juice. Histamine, 100 mg/kg, was given subcutaneously 4 hours after the ulcer, and the ulcer was not apparent to be mediated by any vascular factor. The ulcers induced by histamine did not appear in all the guinea-pigs injected even though lethal doses of histamine were used. 100 mg/kg of H<sub>2</sub> also showed that ulceration did not occur in all the animals.

butazone in order to neutralize the acid and thus determine the ulcerogenic potency of phenylbutazone alone. Since the gastric damage appeared and extended particularly during the first six hours after administration of the phenylbutazone, when the acid concentration was falling from the normal fasting value almost to zero, the alkali was given at hourly intervals during this period and the guinea-pigs were killed at the end of it. The acidity of the gastric contents was examined at the time of death with Topfer's reagent, and the contents were classified as acid if the pH was less than 4.2 by this test.

In the group which received 30 mg of MgO, the incidence of ulceration was reduced in comparison to those which received phenylbutazone alone, and the number of ulcers per animal, in those which developed ulcers, was also diminished. A similar reduction in incidence and degree of gastric damage occurred in the group which received 200 mg of MgO at hourly intervals. No free acid was found in the gastric contents of either group post-mortem, the incidence of ulceration was reduced to about 70%, and the severity of the damage was reduced in comparison with that in the guinea-pigs which received phenylbutazone alone (Table 22).

In the group which received MgO at 1 or 4 hour intervals during a period of 12 hours after the phenylbutazone free acid was found in four out of eight animals and only in one was no gastric damage found. No acid was present in the gastric contents of the animal in which there was no gastric damage. However, there was extensive damage in the other three animals in which no acid was present in the stomach. The gastric damage in the last group was more extensive than in the other two groups which received phenylbutazone.

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The observation of Hay *et al.* (1942) that gastric ulcers can be produced in guinea-pigs by the injection of a histamine-beeswax mixture has been confirmed and it has been shown that this is accompanied by the secretion of a high volume of gastric juice. Histologically, these ulcers resembled phenylbutazone ulcers, and like the latter, did not appear to be mediated by any vascular factor. The ulcers induced by histamine did not appear in all the guinea-pigs injected even though lethal doses of histamine were used. Hay *et al.* (1942) also observed that ulceration did not occur in all their animals.



TABLE 22

The effect of magnesium oxide on the degree and incidence of gastric damage and the acidity of the gastric contents in animals which had received phenylbutazone 200 mg/kg I.P.I.

Treatment	% incidence	No. ulcers per animal	Time after phenylbutazone hrs.	% animals with acid in gastric contents
No alkali	100	36	6	58
Group 1 MgO 30 mg/kg	70	4	6	0
Group 2 MgO 200 mg/kg	66	7	6	0
Group 3 MgO various doses	87	15	12	50

The administration of magnesium oxide caused the disappearance of free acid from the gastric contents when the animals were killed six hours after administration of the phenylbutazone. This does not indicate that there was no free acid in the gastric contents throughout this period, but it is certain that its concentration was very much considerably reduced. The magnesium oxide did not cause the disappearance of the gastric lesions, but did reduce their number and incidence. No difference was observed between those which occurred in the presence of phenylbutazone alone and those which occurred in the presence of phenylbutazone and the alkali. In the gastric-pipe which received the smallest dose of alkali, neutralization of the acid was incomplete and the incidence of ulceration was greater. In the experiments in which the larger doses of magnesium oxide were given, gastric lesions were found in animals which had no free acid in their stomachs at post-mortem. These results make it clear that phenylbutazone can cause gastric ulcers in the presence of acid concentrations much lower than those normally present in the fasting stomach and suggest that in some of the experimental animals, in the absence of

These experimental results clearly show that if a vascular factor plays a part in the production of the ulcers, it is not of importance during the initiation of the lesion. The part which it plays during the later stages appears to be secondary to the superficial necrosis, and is associated with rupture of the vessels in the base of the ulcer and secondary haemorrhage. The remaining factors which might be responsible for the initiation and growth of the ulcers are the high concentration of free acid, and the relatively high output of pepsin in the gastric contents, acting either individually or in conjunction, to produce digestion and subsequent erosions in the gastric mucosa. Code has stressed the importance of the acid in this process. If it is so important, it is difficult to understand why ulcers occur only in about 75% of the guinea-pigs, because at post-mortem the gastric contents always contain a high concentration of acid, both in those which have, and in those which have not got ulcers.

The gastric damage was more severe and appeared more rapidly after the phenylbutazone than after the histamine. Its superficial nature and the appearance of the lesions only at the time when gastric secretion was occurring both suggest that the damaging effect of phenylbutazone was associated with active secretion. The facts that the drug was administered parenterally and that Bonfils *et al.* (1955) have shown that it is not secreted into the gastric lumen, indicate that the damage was not due to any direct toxic effect of the drug on the surface of the gastric mucosa. During the 0 - 6 hour period, it is unlikely that enzymatic activity was high and during the 14 - 24 hour period, it was absent; it may, therefore, be concluded that pepsin played no part in the initiation of the gastric damage. During both these periods, acid was present in the gastric contents and it therefore seemed probable that this was the agent in the gastric secretion which was responsible for the gastric damage.

The administration of magnesium oxide caused the disappearance of free acid from the gastric contents when the animals were killed six hours after administration of the phenylbutazone. This does not indicate that there was no free acid in the gastric contents throughout this period, but it is certain that its concentration must have been considerably reduced. The magnesium oxide did not cause the disappearance of the gastric lesions, but did reduce their number and incidence. No difference was observed between those which occurred in the presence of phenylbutazone alone and those which occurred in the presence of phenylbutazone and the alkali. In the guinea-pigs which received the smallest dose of alkali, neutralization of the acid was incomplete and the incidence of ulceration was greater. In the experiments in which the larger doses of magnesium oxide were given, gastric lesions were formed in animals which had no free acid in their stomachs at post-mortem. These results make it clear that phenylbutazone can cause gastric ulcers in the presence of acid concentrations much lower than those normally present in the fasting stomach and almost certainly in some of the experimental animals, in the absence of

acid. It is therefore suggested that a specific gastric damaging effect is associated with the administration of phenylbutazone or with the production of its metabolic products. This effect is shown by the appearance of superficial necrosis in the gastric mucosa and is exacerbated by the presence of acid in the gastric contents.

The changes in the gastric secretion and the morphological changes which appear after the administration of histamine and phenylbutazone, have been described. In each type of ulcer vascular changes appeared late as a consequence of the superficial mucosal necrosis which formed the initial damage. In the gastric ulcers appearing after both drugs, the histological changes were similar. Hay *et al.* (1942) have pointed out the similarity between histamine induced ulcers and human gastric ulcers; phenylbutazone ulcers also resemble human gastric ulcers in the progress and development of their morphological characters (Mauer 1955). Phenylbutazone differs from histamine in that it produces a large number of severe gastric ulcers, but duodenal ulcers only rarely follow its administration. Histamine causes gastric and duodenal ulcers and the latter occur almost as frequently as the former. Phenylbutazone in the large doses used in the present experiments, caused ulceration in all the animals to which it was administered, whereas, histamine both in the present experiments and in those originally described by Hay *et al.* (1942) produced ulcers in only about 75% of the guinea pigs. The character of the secretion produced by the two drugs also differed very significantly in its digestive ability.

The mechanism of production of the phenylbutazone ulcers clearly differed from the accepted belief about the production of histamine ulcers. Pepsin certainly was not instrumental in causing their production since it was either inactive or absent during the periods when they appeared. It is not possible entirely to exclude the acid present in the canaliculi of the parietal cells or the lumina of the gland tubules from playing any part in their production during the administration of  $MgO$ , but it is extremely improbable that it was present in sufficient concentration to have any destructive effect on the gastric mucosa. This is confirmed by the fact that increasing the dose of alkali from 30 to 200 mg hourly did not cause any reduction in the number of ulcers which appeared. It is, however, clear that the presence of acid in the gastric contents can increase the degree and severity of the gastric damage. The appearance of gastric ulcers morphologically similar to histamine-induced ulcers does not therefore appear to depend on the presence of pepsin or acid in the gastric contents.

Pickering & James (1949) have suggested that gastric and duodenal ulcers are diseases of different pathogenesis. They base this suggestion on the evidence that duodenal ulcer is associated with the prolonged and excessive action of acid, whereas gastric ulcer generally occurs in the presence of normal or low concentrations of acid. The fact that acid may not be

the only factor essential for ulcer formation is also supported by the observation that hypersecretion of acid often continues after the healing of duodenal ulcers. Direct stimulation of the vagus nerves experimentally, or prolonged administration of parasympathetic drugs, both of which cause the secretion of gastric juice containing a high concentration of pepsin and acid, may produce mucosal haemorrhages and superficial gastric ulceration, but not chronic peptic ulcers resembling those produced by histamine (Best & Orator 1933; Ettinger, Hall & Banting, 1936). This evidence suggests that although acid may be an important factor in accelerating the progress of ulcers after the original lesion has occurred, the initial fall in the resistance of the gastric mucosa does not depend upon the destructive or digestive action of the acid in the gastric contents. It may, therefore, be concluded that endogenous histamine in virtue of its action in stimulating the secretion of acid by the parietal cells is not responsible for causing the initial weakness in the gastric mucosa which subsequently develops into an ulcer. The stimulant action of histamine however, is responsible for the progress of the ulcer in its later stages.

It is suggested therefore, that the greater activity of gastric cells, the more histamine is secreted, and the more histamine is secreted, the more acid is secreted. This conclusion is supported by the observation that a large proportion of the histamine secreted is absorbed by the liver, liver, striated muscle and then, some of which is secreted into the blood. It is suggested therefore, that the greater activity of gastric cells, the more histamine is secreted, and the more histamine is secreted, the more acid is secreted.

Extensive histamine is not absorbed in the small intestine, but is absorbed by the skin in the dog and rat. However, a similar observation with regard to histamine absorption in these mammals together suggest that very little histamine is absorbed in the small intestine. Since most of the histamine is absorbed in the skin, it follows that histamine is not absorbed in the small intestine.

It is clear that a proportion of exogenous histamine, whether administered by mouth or parenterally, is rendered physiologically inactive. It is probable that the histamine is taken up by the tissues in which the histamine liberators have relatively little effect, such as the kidney, striated muscle, and liver, with the exception of the liver, which is not affected. In the dog and rat, the loss from the small intestine and other organs would be reduced, the normal turnover of histamine in the body would be reduced, and the inactivation of exogenous histamine might be more rapid because the inactivating enzymes would be more available. In such circumstances, after the initial loss of histamine from the stores, depleted animals would be as resistant, if not more resistant, than normal animals to exogenous histamine.



## CHAPTER 6

### THE ROLE OF HISTAMINE IN LIVING TISSUES

It has been shown that exogenous histamine is rapidly absorbed from the blood and metabolized by the tissues; the physiologically inactive form is excreted with a small proportion of the active form. The uptake and destruction of the physiologically-active histamine by all the tissues allows of its rapid disappearance from the blood and thus reduces the intensity and duration of its pharmacological action. The kidney in the rat is particularly effective in destroying histamine and its inactivating ability is clearly much more important than its ability to excrete histamine in the urine. Histamine is absorbed and destroyed by other tissues, including the liver, intestine and skin. Schayer (1956b) has shown that exogenous histamine is not bound by the tissue mast cells; he considers that their histamine is made by the decarboxylation of histidine and that the product is then bound. If this is true, the exogenous histamine which entered the tissues in the present experiments did not enter the mast cells. This conclusion is supported by the observations that a large proportion of the exogenous histamine entered the kidney, liver, striated muscle and ileum, none of which contains many mast cells. It is suggested therefore, that the uptake and metabolism of exogenous histamine depends upon the activity of cells other than the mast cells in the normal animal.

Exogenous histamine is not absorbed to any significant extent by the skin in the depleted rat. Schayer & Smiley (1954) made a similar observation with regard to radio-active histamine. These observations together suggest that very little inactivation of physiologically-active histamine occurs in the skin. Since most of the histamine in the skin is held in the mast cells, it follows that inactivation of the exogenous histamine does not occur in the mast cells.

It is clear that a proportion of exogenous histamine, whether administered by mouth or parenterally, is rendered physiologically inactive. It is probable that the histamine is taken up by the tissues in which the histamine liberators have relatively little effect, such as the kidney, alimentary canal, and liver, with the exception of the liver capsule, and metabolised there in the normal fashion. In depleted rats, the loss from the mast cells and other stores would be reduced; the normal turnover of histamine in the body would be smaller, and the inactivation of exogenous histamine might be more rapid because the inactivating enzymes would be more available. In such circumstances, after the initial loss of histamine from the stores, depleted animals would be as resistant, if not more resistant, than normal animals to exogenous histamine.

The role of endogenous histamine has been investigated in two pathological states. However, its role in these states differs in important respects and the methods of investigation had to be varied accordingly. The effects of irradiation could have been caused by the local action of intrinsic histamine and by the general symptoms produced by the action of extrinsic histamine. Methods of investigation were, therefore, used to determine whether the local lesions could be prevented when histamine was absent from local sites, and whether there was a generalised release of histamine which might account for the general toxic symptoms of the radiation syndrome. Gastric ulcer is a common pathological condition in man which can be reproduced easily in experimental animals by the injection of histamine. Circumstantial evidence suggests that it occurs as a result of the pharmacological action of histamine and in order to prove or disprove this, a method of producing ulcers was evolved in which the acid-stimulating action of histamine was reduced to a minimum, while ulcers were appearing. By this means, the extent to which histamine was responsible for producing the ulcers could be assessed. The methods used in these investigations differed from each other in that the tissue concentrations and metabolism of histamine were of primary importance in the first, and the pharmacological actions of histamine were the factors on which the second set of investigations depended.

Irradiation caused damage in the skin and in the alimentary canal. In both tissues it caused the break-up of mast cells and consequent changes in the normal metabolism of histamine. The pathological changes in the skin occurred when the intrinsic histamine had been reduced to a minimal and probably insignificant value. The damage in the alimentary canal was also not associated directly with the changes in its intrinsic histamine. Histamine liberators do not cause damage resembling that produced by irradiation in either of these tissues. A liberator on account of its extensive destruction of mast cells can, however, release a much larger quantity of histamine, more suddenly, than irradiation is able to do in the skin. This suggests that irradiation causes the damage independently of the release of histamine. Irradiation differs from all the histamine-liberators in releasing histamine from the alimentary canal; thus it may be concluded that irradiation produces its effects on the tissue histamine by a different mechanism than that of the histamine liberators. Examination of the tissues when histamine release occurred, suggested that irradiation caused its effects not only by releasing histamine from the tissue stores but also by interfering with the enzymes which formed and bound the histamine in the tissues, particularly in the alimentary canal. It is possible that irradiation might also interfere with the activity of the histamine-catabolizing enzymes. In this way, its action would differ from that of the histamine-liberators in that irradiation would then delay the rate of disappearance of histamine from the blood.

Mongar & Schild (1957, 1958) have shown that the release of histamine is the last of a train of processes which occur in the anaphylactic reaction and, although it is responsible for some of the signs and symptoms of the reaction, it is of little importance when the mechanism of the early part of the reaction is being considered. The mechanism of production of radiation lesions may depend upon a similar type of enzymic reaction in which the release of histamine is a terminal stage. Ungar & Damgaard (1954a) have shown that part of the proteolysis which occurs in the thermal injury is caused by enzymatic action and have suggested that a similar system may be activated in radiation lesions (1954b). If a tissue protease is activated by irradiation, and is responsible for the initial tissue injury, it is understandable why the tissue injury can occur in the absence of histamine. If a chain reaction, similar to that described by Mongar & Schild occurs, histamine would be released after the signs of the initial damage had appeared, as was found to be the case in these experiments.

Irradiation released histamine by causing the break-up of tissue mast cells and possibly by initiating a process of cell damage of which one of the end results was the release of intrinsic histamine. Weber & Steggerda (1949) described an increase in the plasma histamine concentration in rats during the first 2 hours and on the fifth day following irradiation, and Leitch & Haley (1955) state that a twofold increase in the urinary excretion of histamine occurred in rats during the post-irradiation period. Venters & Painter (1950) have shown in the rabbit and dog, that there is increased sensitivity to the infusion of histamine following irradiation and have suggested that the radiation syndrome might partly be produced by the liberation of a toxic product from the mucous membrane of the small intestine. The increased sensitivity to infusion of histamine which they report would be understandable if the function of the enzymes which inactivate histamine was disturbed following irradiation, and the toxic product which they found might have been the intrinsic histamine which is released from the intestine. The sequence of the decreases in the tissue histamine which was observed in the present experiments, first in the alimentary canal and then in the skin, corresponds to the fluctuations in plasma histamine reported by Weber & Steggerda (1949), and provides an explanation for the source of the increased urinary excretion of histamine described by Leitch & Haley (1955).

Although the histamine is released gradually after irradiation, in comparison with the sudden release following a histamine liberator, a large quantity must be released from the tissues into the blood after irradiation. In a normal animal it would probably be sufficient to cause some signs. In irradiated animals the action of the metabolising enzymes in the tissues may be disturbed, and the disappearance of the released histamine from the blood may take longer than normal. The



release of the intrinsic histamine may thus be responsible for the general toxic effects of the radiation syndrome although it is not the agent which causes the local tissue damage. It is clear that the mast cells serve principally as local tissue stores of histamine and that they do not inactivate physiologically-active histamine. Histamine itself appears to play no part in producing the local tissue damage associated with irradiation. In order to examine the mechanism of production of this damage, and to control the release of histamine, the most effective line of research will be the investigation of the local enzymes in the tissues which control proteolysis.

In the last chapter, it was shown that neither high concentrations of acid or pepsin in the gastric secretion, nor vascular factors in the gastric mucosa, are responsible for the initial lesion which subsequently develops into a gastric ulcer. The principal action of histamine in the stomach is believed to be the stimulation of the secretion of acid and therefore, it seems unlikely that histamine can be involved in the initiation of gastric ulceration. High doses of histamine and phenylbutazone were administered in the experiments described in Chapter 5, and it might be suggested that both these drugs caused the ulcers by specific toxic effects. This is unlikely to be the mechanism by which histamine caused the ulcers, since it is difficult to produce ulcers in all the guinea-pigs even when using lethal doses of the drug. The gastric mucosa of some of the animals is apparently insusceptible to any toxic effects which the histamine may have. Also, it has been shown in Chapter 3 that histamine is metabolised and excreted extremely rapidly, yet gastric ulcers rarely appear much sooner than 12 - 24 hours after administration of the histamine. The doses of phenylbutazone administered in the experiments which have been described were not as large as they may appear to be when compared with the therapeutic dose administered to man. Brodie (Brodie & Hogben, 1957; Burns *et al.* 1953; Burns *et al.* 1955), has shown in man that 98% of the drug is bound to the plasma proteins and that its biologic half-life is about 72 hours. In the guinea-pig and rat, the half-life of the drug is 5 - 6 hours and the plasma binding is about 85%. It is clear that the drug is metabolised rapidly in the guinea-pig on account of the diminished plasma binding, and thus in order to maintain plasma concentrations in this animal which allow it to have comparable pharmacological effects to those in man, a much larger dose must be given to the guinea-pig. Domenjoz (Domenjoz, 1952; Wilhelmi & Domenjoz, 1951) has found that a dose of 100 - 200 mg/kg produces comparable pharmacological effects in the guinea-pig to those produced therapeutically in man. The large number and variety of drugs which can produce gastric damage, including histamine, phenylbutazone, salicylates, cinchophen and ACTH, make it most improbable that any common damaging effect of the drug molecules on the gastric mucosa is responsible for the gastric lesions. It is, therefore, suggested that the common damaging effect of the histamine and phenylbutazone on the gastric mucosa



must be attributed to some common effect on the constitution of the gastric secretion.

This conclusion clearly indicates the necessity of investigating the gastric secretion in order to discover a constituent, the concentration of which is related to the appearance of gastric damage. Inhibition of the secretion of a "protective" or "anti-ulcer" substance such as urogastrone or antheilone (Sandweiss 1945) or of the polysaccharide of the specific blood group 'A' substance (Clarke *et al.* 1956) might be responsible for causing the initial weakness in the gastric mucosa. The presence of acid in the gastric contents would then increase the severity of the initial mucosal lesion produced by lack of the protective factor. Differences in the severity of the gastric damage produced by drugs such as phenylbutazone, histamine or methacholine would become explicable primarily in terms of their effects on the concentration of the protective factor in the gastric secretion, and secondarily in terms of their effects on the digestive action of the gastric juice.

The polysaccharide of the specific Group 'A' substance exists in the gastric mucosa of the guinea-pig (Pasternak *et al.* 1958) and is known to be associated with the gastric mucin (Morgan & King, 1943), which disappears in the early stages of development of drug induced ulcers. For these reasons, it was felt that investigation of this substance and the factors which control it in the gastric secretion would be of more value in the investigation of the initial weakness in the gastric mucosa, which subsequently develops into an ulcer, than further study of the factors which control the secretion of acid in the stomach. By using human and anti-guinea-pig rabbit serum, blood group substances resembling human 'A' and 'B' blood group substances have been found in the gastric contents in concentrations which show little variation from animal to animal. It has been found that histamine causes a reduction in their concentration six hours after injection of the histamine in about a half to two-thirds of the animals, and that methacholine causes an increase in the concentration of these substances in the gastric secretion. These preliminary experiments suggest that blood-group substance, of a nature similar to that in human saliva, exists in the gastric contents of the guinea-pig, and that its concentration is reduced by an ulcerogenic drug and is unaffected or increased by a drug which does not produce ulcers. These observations, although still very far from being complete, do give some evidence of a positive nature for the mechanism by which the initial weakness in the gastric mucosa may arise, and suggest a way in which the field of research may be extended.

Histamine is absorbed and rapidly metabolised by the tissues so that the extent to which it is responsible for general toxic effects in the body is limited. The mast cells are depots for the storage of histamine in the tissues but do not take up the physiologically-active compound or inactivate it. It

has been shown that histamine is released as a result of irradiation of the tissues but that it is not responsible for the local tissue damage. In the stomach, it is responsible for extending the gastric ulcer but does not cause the initial weakness in the mucosa from which the ulcer develops. The experiments which have been described have shown that histamine is not primarily responsible for the disintegration of tissue resistance. However, after the biochemical lesions have occurred, which initiate the tissue changes, histamine increases the severity of the damage by virtue of its general toxic effects, or of its more local secretory effects in the stomach; the original damage can occur in the absence of any action by endogenous histamine. It is clear that the role of endogenous histamine in the production of tissue damage is of a secondary rather than a primary nature.

Research in any field is constantly progressing and extending. In the introduction, it was pointed out that the tissue metabolism of histamine, the mechanism of production of radiation lesions, and the initiation of ulcers in the gastric mucosa, might all be related by the physiological and pathological turnover of histamine. The experiments which have been described, show that endogenous histamine plays only a minor part in initiating the lesions and that its later role is not important. The evidence from these experiments suggests that the focus of the research should be shifted from the action of endogenous histamine as its main point, to tissue enzymes and blood group substance. At present, these appear to be unrelated, but as more is learnt about them, some common trait may appear. A substance which has a protective action in the stomach may be related to a tissue enzyme affecting the resistance of the skin against radiation damage, or preventing the break-down of the mucosa of the intestines. Knowledge of the details of the uptake and metabolism of histamine by the tissues is of importance for the analysis of the general symptoms associated with pathological processes, but the role of intrinsic histamine in the production of local tissue lesions has been shown to be of less importance as more is being learnt about the facts which determine the sequence of events in the tissues.

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